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Expedient Methods for the Stereoselective Glycosylation of Carbohydrates

Ryan Williams

A dissertation submitted to the University of Bristol in accordance with the requirements for award of the degree of Doctor of Philosophy in the Faculty of Science

School of Chemistry

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Supervisor: Prof. M. Carmen Galan

67,630 words

Abstract

Carbohydrates are a widely abundant class of biological compounds that play pivotal roles in medicine, cellular function and nutrition. Despite the ubiquity of carbohydrates in living systems, their efficient synthesis presents a significant challenge. Major reasons for this include the difficulties in controlling the stereochemistry of the glycosylation reaction and the laborious, time-consuming nature of oligosaccharide preparation. This thesis describes efforts to overcome these challenges through new technologies that expedite glycosylation reactions and subsequent product purification.

A library of glycosyl donors and acceptors was prepared. Using these substrates, a cooperative thiourea-Brønsted acid dual organocatalytic glycosylation strategy for the synthesis of 2-deoxyglycosides was probed. Experiments were performed to uncover mechanistic information about the organocatalytic reaction. Subsequently, a palladium catalysed glycosylation protocol for the preparation of 2-deoxyglycosides was developed. After optimisation of the model reaction, the tolerance of the method for various glycosyl acceptors was assessed through a substrate screen. Generally, high product yields were obtained with excellent α selectivity. A probable mechanism is discussed.

Continuous flow glycosylation reactions were then explored. Initially, a flow glycosylation protocol using a gold(I) catalyst was surveyed. Some promising results were discovered, however, the system proved inconsistent and ultimately unsuitable for the flow regime. Ionic liquid supported glycosylations in flow were then explored for the first time. After reaction optimisation, excellent yields of glycosylation product were obtained in just 15 seconds residence time, with no requirement for column chromatography. Application of the optimal reaction conditions to several other glycosyl donors and acceptors proved challenging due to unexpected protecting group reactivity leading to side products and irreproducibility of results. Nonetheless, mixtures of β-1,2-glucans were successfully prepared using this method, whilst a novel glycosyl donor featuring an orthogonal carbonate protecting group was identified as a key scaffold for the synthesis of β-1,6-glucans.

Author’s Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: ..... DATE:.....

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My gratitude also goes out to the School of Chemistry and the University of Bristol as a whole. It is an incredible, world-class institution for learning, research and innovation that has allowed me to develop into a scientist by providing the resources, knowledge and ethos

for success. I will carry the skills and experiences I acquired here for the rest of my life. In the same vein, I would like to thank the people and institutions that comprise the city of Bristol. When considering whether to start a PhD here, I had a single day in the city to decide whether to live here for the next four years. The decision I made seems to have been the right one, as I have come to love and value the city and everything that makes it such a rich, colourful and vibrant place to live.

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Table of Contents

1. Abbreviations

Ac	Acetyl	**M**	Molar
AIBN	Azobisisobutyronitrile	**MALDI**	Matrix Assisted Laser Desorption/Ionisation
BINOL	1,1'-Bi-2-naphthol	**min**	Minutes
Bn	Benzyl	**MS**	Mass Spectrometry
Boc	*tert*-Butyloxycarbonyl	**ms**	Molecular sieves
bpy	2,2'-Bipyridine	**^{n}Bu**	*n*-Butyl
Bu	Butyl	**NBS**	*N*-Bromosuccinimide
Bz	Benzoyl	**NCS**	*N*-Chlorosuccinimide
^{13}C	Carbon-13	**NIS**	*N*-Iodosuccinimide
CAN	Ceric ammonium nitrate	**NMR**	Nuclear Magnetic Resonance
Cbz	Benzyloxycarbonyl	**Nu**	Generic nucleophile
DABCO	1,4-Diazabicyclo[2.2.2]octane	**P**	Promoter
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene	**PG**	Protecting group
DCM	Dichloromethane	**Phth**	Phthalimido
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone	**PMP**	*para*-Methoxyphenyl
DMAP	4-Dimethylaminopyridine	**PTFE**	Polytetrafluoroethylene
DMAPA	*N,N*-Dimethylaminopropylamine	**Pyr**	Pyridine
DMF	Dimethylformamide	**R**	Generic residue
DTBMP	2,6-Di-*tert*-butyl-4-methylpyridine	**R_f**	Retention factor
eq	Equivalents	**RT**	Room temperature
ESI	Electrospray ionization	**s**	Seconds
Fmoc	9-Fluorenylmethoxycarbonyl	**TBAF**	*tert*-Butylammonium fluoride
^{1}H	Proton	**TBDMS**	*tert*-Butyldimethylsilyl
h	Hours	**TBDPS**	*tert*-Butyldiphenylsilyl
HPLC	High Performance Liquid Chromatography	**^{t}Bu**	*tert*-Butyl
HRMS	High Resolution Mass Spectrometry	**Tf**	Trifluoromethanesulfonyl
ICROS	Ionic Catch and Release Oligosaccharide Synthesis	**TFA**	Trifluoroacetic acid
IDCP	Iodonium dicollidine perchlorate	**THF**	Tetrahydrofuran
IL	Ionic Liquid	**TIPS**	Triisopropylsilyl
^{i}Pr	Isopropyl	**TLC**	Thin Layer Chromatography
IR	Infra-red	**TMS**	Trimethylsilyl
L	Ligand	**Tol**	*para*-Methylphenyl
LA	Lewis acid	**Troc**	2,2,2-Trichlorethoxycarbonyl
LED	Light Emitting Diode	**Ts**	*para*-Toluenesulfonyl
Lev	Levulinyl	**X**	Generic group
LG	Leaving group		

2. Introduction

Section **2.1.** of this introduction will outline the ubiquity and chemistry of carbohydrates and the individual monosaccharide subunits that comprise them, as well as contextualising carbohydrates and glycoscience through a brief examination of some of the biological and medicinal roles that carbohydrates play. Section **2.2.** will then go on to examine the glycosylation reaction in which a glycosyl donor and acceptor are coupled, including the principles behind traditional routes to stereochemical control and some factors that affect reactivity of the coupling partners. In section **2.3.** 2-deoxyglycosides will be introduced. Attention will be paid to glycals as glycosyl donors for 2-deoxyglycoside synthesis, then some existing routes to 2-deoxyglycosides will be outlined. In section **2.4.** a modest examination of the applications of transition metal catalysis to the glycosylation reaction will be undertaken, including examples of 2-deoxyglycoside synthesis using transition metal catalysts. Conversely, section **2.5.** will outline some organocatalytic glycosylation approaches. Section **2.6.** will focus on shedding light upon the emerging scope for glycosylation through flow methodology by examining some recent literature reports, whilst section **2.7.** will briefly study the use of ionic liquid tags as supports for the expedition of oligosaccharide synthesis.

2.1. Carbohydrates

Carbohydrates represent the most plentiful type of natural products on the planet, exhibiting immense structural diversity and myriad biological functionality.[1-8] Oligosaccharides have been established as being an essential component for various biological processes including inflammation, neural development, cell signalling, protein folding, fertilisation and embryogenesis.[4] Moreover, complex naturally occurring glycans play a crucial role in the development and proliferation of various diseases, since carbohydrates are known to be involved in biological events such as immune response, bacterial adhesion, viral infection and cancer. These roles make carbohydrates valuable target compounds for the diagnosis, comprehension and treatment of disease.[9-14]

Carbohydrates are a group of natural products that are so named because the simplest carbohydrates have the empirical formula $C_n(H_2O)_n$, literally hydrates of carbon. However,

this empirical formula does not account for the wide variety of compounds encompassed within the broad terminology of "carbohydrate" that do not adhere to this formula. Monosaccharides are the simplest carbohydrate units from which all other carbohydrates are built. They may be joined into chains of two monosaccharides (disaccharides), roughly 3-10 monosaccharides (oligosaccharides) or up to thousands of monosaccharides (polysaccharides) through glycosidic linkages. Monosaccharides take many forms but are generally variations on the theme of a 5 or 6-membered heterocyclic ring featuring an endocyclic oxygen atom (**Figure 1**). Each monosaccharide contains at least one carbonyl moiety and several hydroxyl groups, giving rich stereo- and regiochemical diversity to even simple carbohydrates. Furthermore, the word "carbohydrate" also describes chemicals derived from monosaccharides through transformations including reduction or oxidation of terminal carbonyl groups and molecules in which hydroxyl groups have been replaced with other functional groups including H atoms, amines, thiols and amides.

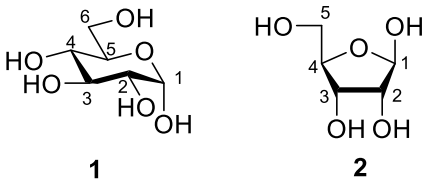


Figure 1. *The structures of two common monosaccharides: α-D-glucopyranose* ***1*** *and β-D-ribofuranose* ***2****, with carbon atoms numbered in the conventional manner.*

In order to highlight the importance of carbohydrates and justify their study, one might look to some poignant examples that showcase their relevance. In the 14th century, the malady bubonic plague swept through Europe, killing an estimated 100 million people within just eight years, around half of the population of the continent at the time. The causative microbe responsible for this devastation is now known to be the bacteria *Yersinia pestis*.[15, 16] In the rare cases of plague that occur today, the first line of defence is generally the amino glycoside streptomycin **3** (**Figure 2**), a carbohydrate derived antibiotic that destroys bacteria through inhibition of protein synthesis.[17] This is generally sufficient to prevent serious harm from the infection. One might consider the dire consequences of antibiotic resistance[18] in bacterial strains in this light and reflect upon the implications of a lack of comprehension of the essential medicinal role of carbohydrates.

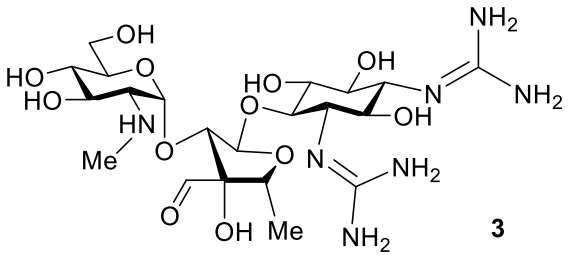
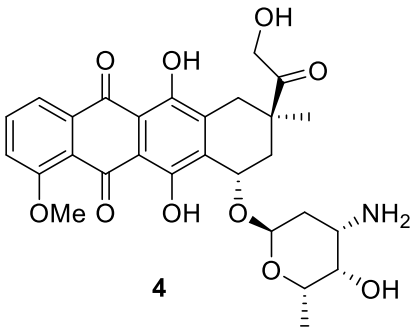


Figure 2. *Carbohydrate derived antibiotic streptomycin **3**.*

Another insidious ailment that is a major concern in the 21st century is cancer. In the U.K., 977 people are diagnosed with cancer every day, whilst 446 die from cancer each day.[19] Furthermore, half of the people born after 1960 are predicted to be diagnosed with some form of cancer in their lifetime.[19] Thus, the requirement for effective therapeutics to treat cancer and prevent these fatalities is paramount. Here again, carbohydrates may offer opportunities to save lives through an appreciation of their biological significance and functional role. Several cancer treatment drugs feature a sugar moiety intrinsic to the function of the drug. Perhaps the most well-known example is doxorubicin **4** (**Figure 3**), an anthracycline that has been a staple chemotherapy agent for some 30 years.[20] Moreover, carbohydrates may be used not only as drugs for cancer treatment, but also as cancer vaccines.[21] For instance, the Globo-H cancer vaccine, originally developed for treatment of metastatic breast cancer but applicable to a number of different cancer types, contains an oligosaccharide moiety that encourages immunological response to cancerous cells.[12] Whilst carbohydrate derivatives can be used as effective drugs,[22] relatively few of the therapeutics currently available contain carbohydrate motifs, owing primarily to the pharmacokinetic disadvantages of these molecules.[23] Orally administered carbohydrates suffer poor transit through the small intestine wall and are often rapidly excreted renally. Nonetheless, carbohydrates represent an under-explored source of new therapeutics.



4

Figure 3. *2-Deoxyglycoside containing anticancer drug doxorubicin* ***4****.*

Carbohydrates are the products of photosynthesis and thus have a major role as a form of chemical energy storage, with billions of tons being produced by plants and cyanobacteria every year.[8] The energy stored within carbohydrates forms perhaps the most important part of the biological food chain for many animals. Indeed, the role of carbohydrate foodstuffs with respect to nutrition, healthy diet and resultant disease prevention in humans is an area of ongoing research.[24, 25] Carbohydrates serve further roles as both constituents of cell walls and as structural features in the supporting tissue of plants and animal shells.[7] The carbohydrates expressed as part of the glycocalyx on the surface of human cells are used to regulate healthy cellular function.[9]

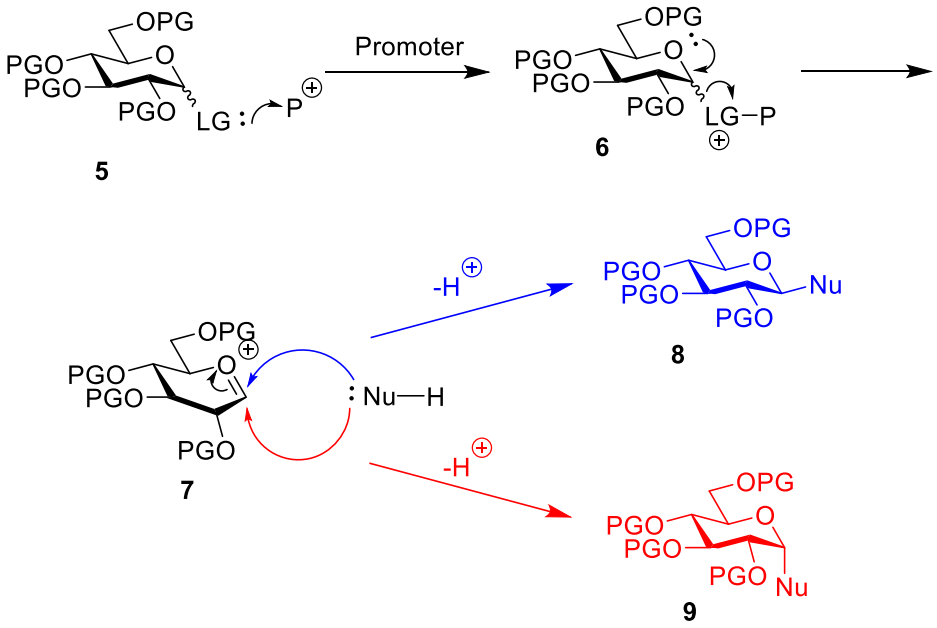
It should therefore be apparent that continued exploration of the function, prevalence and applications of carbohydrates is crucial. Whilst the research presented in this thesis comprises only a minute area of glycoscience, it is nonetheless important to recognise the purpose of these studies in a larger context and be mindful of the distant, lofty, but incrementally attainable goals and applications of this work.

2.2. **Glycosylation**

In order to study the role that carbohydrates play in biology, adequate quantities of pure, structurally defined carbohydrates must be prepared. For the synthetic glycoscientist, a key reaction is that of glycoside bond formation. Numerous glycosylation strategies exist that utilise a wide variety of glycosyl donors, acceptors, promoters and catalysts, both chemical[26-36] and enzymatic.[37-42] Despite each methodology having distinct advantages, at present there is no universal glycosylation strategy that can give access to all required

oligosaccharides with consistently high levels of stereoselectivity and efficiency. Hence, more advanced protocols for glycosylation are imperative in order to prepare the carbohydrates found in nature on a reasonable scale and with high purity and thus advance our comprehension of carbohydrate function.

Glycosylation is the name given to the process by which a carbohydrate (the glycosyl donor) is linked to a functional group, often a hydroxyl group, of another molecule (the glycosyl acceptor).[4] Whilst nature has evolved enzymes that catalyse glycosylations with excellent stereo- and regiochemical control, the substrate scope of enzymatic glycosylations can be quite limited owing to the specific conformational requirements of the enzyme active site. An alternative is chemical glycosylation, in which the reaction conditions are much less specific to particular reactants, but control of the stereo- and regiochemistry during the reaction is often challenging. Consider the general glycosylation shown in **Scheme 1**.



Scheme 1. *Scheme showing the standard strategy for a chemical glycosylation and the problem of stereochemistry at the anomeric carbon (C-1).*

General electrophilic glycosyl donor **5** forms a donor-promoter complex **6** upon reaction with a promoter reagent, which then undergoes loss of the leaving group (LG), to irreversibly generate cationic oxocarbenium ion **7**. Recent studies by Blériot[43] and co-workers have proven by NMR spectroscopy the existence of the highly reactive oxocarbenium ion in

a condensed phase by stabilising it under super-acidic conditions. This would imply that glycosylation reactions advance via a unimolecular S_N1 mechanism. However, chemical glycosylations are usually performed under much milder conditions than those used by Blériot and co-workers and hence it is impossible to confidently discount an S_N2 or S_N2-like mechanism.[44-46] Furthermore, the tendency for the reaction to progress through an S_N1 or S_N2 type mechanism will be influenced by the complex specific reaction conditions, with variables including glycosyl donor and acceptor, promoter/catalyst, additives, reaction temperature and solvent affecting the mechanism, making a clear distinction between S_N1 and S_N2 type mechanisms difficult. Nonetheless, today it is generally assumed that glycosylation reactions prefer to progress via an S_N1 type mechanism.[45]

The nucleophilic glycosyl acceptor Nu may attack on either the "top" face (blue pathway) or "bottom" face (red pathway) of oxocarbenium ion **7** to generate a glycosidic bond. These two pathways lead to two diastereomers, the equatorial anomer **8** and axial anomer **9**. For all sugars in the D-series the axial anomer is designated the α anomer, whilst the equatorial anomer is designated β by convention. These two diastereomeric species will tend to exhibit different behaviour in biological systems and can be very difficult to separate by conventional chemical purification methods such as chromatography, trituration or recrystallisation. Thus, successful glycosylation approaches will favour the formation of one anomer over the other, ideally forming a single anomer exclusively. Note that protecting groups must generally be installed on all free nucleophilic functional groups on the glycosyl donor (and acceptor if applicable), otherwise any free nucleophile may act as the glycosyl acceptor, leading to a complex mixture of products. Over the years, a great many glycosylation strategies have been developed to meet the challenge of stereoselectivity during glycosylation.[30, 32, 34, 47, 48]

A key factor dictating the stereochemical outcome of the glycosylation reaction is the anomeric effect. This effect describes the higher than expected tendency for formation of the axial anomer in glycosylations. Generally, substituents on a cyclohexane derivative in a chair conformation prefer to adopt an equatorial position to minimise steric repulsion. The anomeric effect states that when the anomeric substituent is a polar group, for instance an alkoxy group or a halide, there is a thermodynamic preference for the axial rather than equatorial configuration, despite the unfavourable 1,3-diaxial steric repulsions this atomic

configuration experiences. Note that more specifically, this effect is known as the *endo* anomeric effect, not to be confused with the gauche effect (sometimes called the *exo* anomeric effect).

Various explanations have been put forward to explain the anomeric effect (**Figure 4**).[8, 32] The first proposal (**A**) concerns the dipole moment of the endocyclic oxygen lone pairs and the dipole moment of the anomeric C-X bond, where X is an electronegative atom. In the equatorial β anomer the dipole moment that points along the C-X bond partially aligns with the O lone pair dipole moment, causing a repulsive destabilisation. Conversely, in the axial α anomer, the C-X bond dipole moment points away from the O lone pair dipole moment, partially neutralising the two dipoles and thus stabilising this configuration. Scenario **B** shows a very similar theory; however, this proposal suggests that it is the dipole moment caused by the lone pairs on atom X, rather than the C-X bond, that is responsible for the anomeric effect. The relative weighting of contribution to the dipole moment between the C-X bond and the X lone pairs likely depends upon the identity of atom X, amongst other factors. Perhaps the most convincing explanation is the stereoelectronic effect that molecular orbital theory describes (**C**). In the case of the axial anomer, the non-bonding lone pair n orbital on the endocyclic O atom can overlap with the C-X σ* antibonding orbital. This hyperconjugation allows delocalisation of the non-bonding electrons and hence lowers their energy. In the case of the equatorial anomer, such orbital overlap is not possible, since the n and σ* orbitals do not have the required antiperiplanar relationship. This explanation is further strengthened by the observation of a shorter endocyclic O-C1 bond and longer C1-X bond in the axial anomer, but not the equatorial anomer.[8]

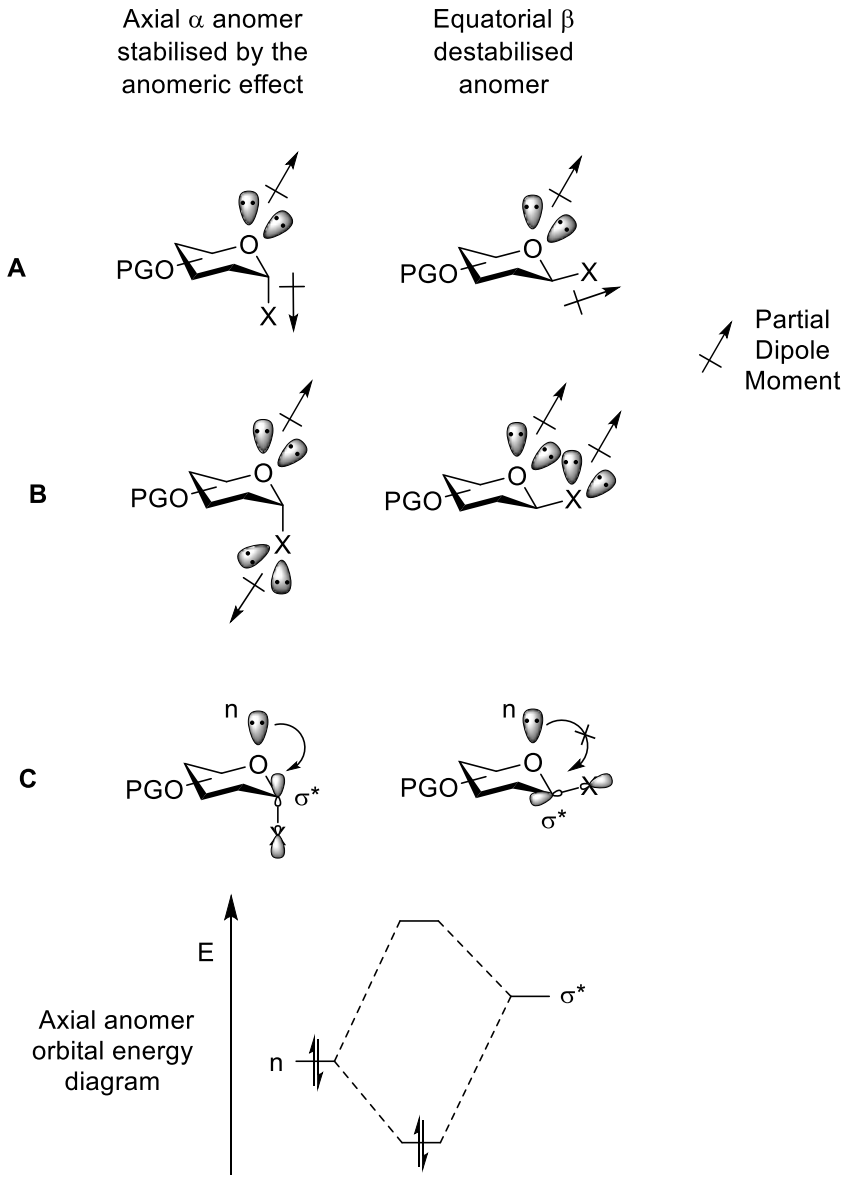
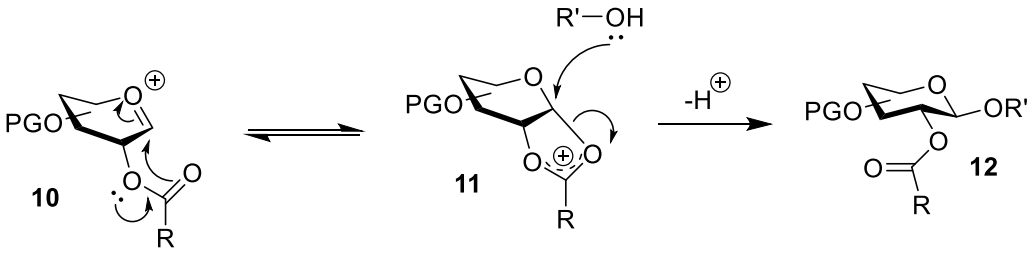


Figure 4. *Different proposed explanations for the anomeric effect.* ***A.*** *Partial dipole moments of the endocyclic O atom lone pairs and C-X bond.* ***B.*** *Partial dipole moments of the endocyclic O atom lone pairs and X lone pairs.* ***C.*** *Hyperconjugation allows delocalisation of O non-bonding electrons into C-X σ* orbital in the axial anomer but not the equatorial anomer.*

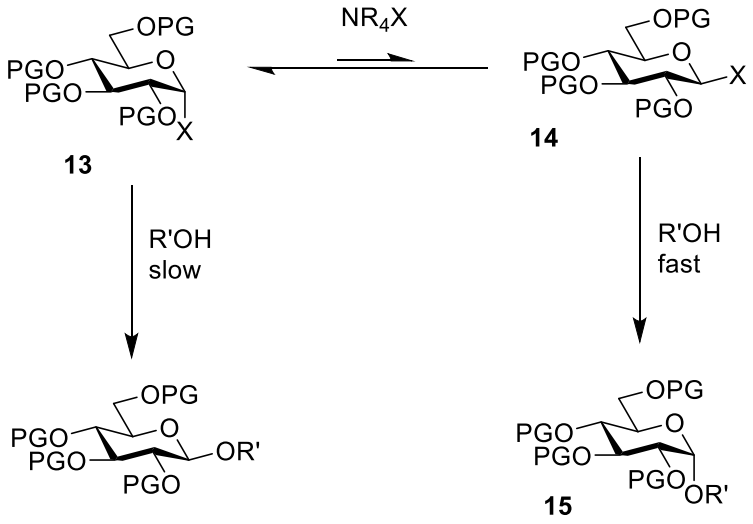
Synthesis of 1,2-*trans* glycosides can be accomplished using neighbouring group participation, otherwise known as anchimeric assistance. This involves the use of a C-2 protecting group that can react intramolecularly with an oxocarbenium ion. This protecting group will generally be an ester, or in the case of 2-amino saccharides, an amide or carbamate. A general synthesis of a 1,2-*trans* glycoside is shown in **Scheme 2**. *O*-2 ester protected oxocarbenium ion **10** is formed by loss of a leaving group as in **Scheme 1**. However, the ester

group can spontaneously form dioxolenium ion **11** by formation of a 5 membered ring, thus blocking the *cis* face of the sugar ring. The attacking glycosyl acceptor alcohol R'OH is then restricted to attack at the *trans* face, forming 1,2-*trans* glycoside **12** as product.



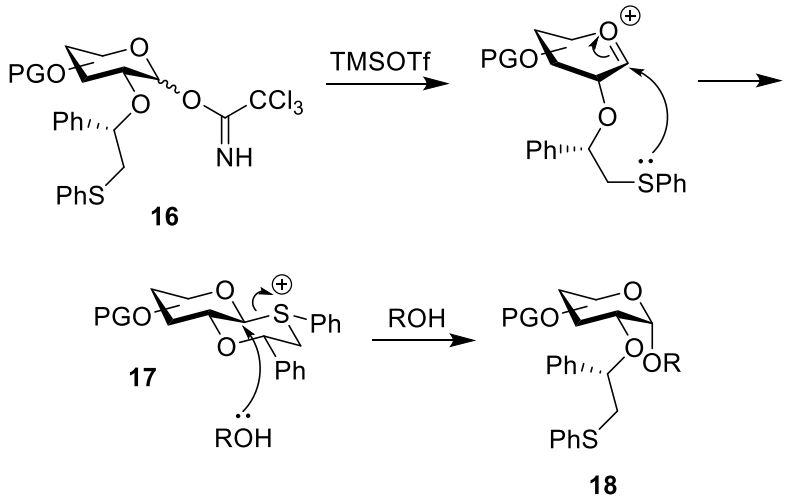
Scheme 2. *Neighbouring group participation as a strategy in 1,2-trans glycosylation.*

The synthesis of 1,2-*cis* glycosides presents a greater challenge, although there are now a multitude of approaches available.[36] One of the first successful approaches was the "*in situ* anomerisation" strategy developed by Lemieux,[49] in which an α-halo glycosyl donor **13** is anomerised to the more reactive β-halo glycosyl donor **14** *in situ* using a tetraalkylammonium halide salt, NR_4X. Fast reaction of the β-halo glycosyl donor with an acceptor in an S_N2 type mechanism furnishes the 1,2-*cis* glycoside **15** (**Scheme 3**).



Scheme 3. *The principles underlying 1,2-cis glycosylation by in situ anomerisation.*

Other approaches include neighbouring group participation using a chiral auxiliary as the C-2 protecting group as demonstrated by Boons and co-workers (**Scheme 4**).[31] Activation of trichloroacetimidate donor **16** creates short-lived *trans*-decalin type β-sulfonium structure **17**, blocking the *trans* face from attack. The incoming nucleophile is forced to approach from the α face, giving the α 1,2-*cis* glycoside **18** selectively.



Scheme 4. *Formation of β trans-decalin structure **17** blocks attack of a glycosyl acceptor on the β face, encouraging α selectivity to give a 1,2-cis glycoside.*

Additionally, temperature and solvent choice can affect selectivity, with ethereal solvents participating to favour 1,2-*cis* glycosides.[47] One may also exploit hydrogen bond mediated aglycone delivery, metal coordination or indirect glycosylation as strategies to facilitate preparation of 1,2-*cis* glycosides.[36]

2.2.1. **Protecting Group Influence on Glycosyl Donor Reactivity**

Research has uncovered stark differences in glycosyl donor reactivity dependent upon the protecting groups chosen to protect the hydroxyl substituents around the monosaccharide ring.[50-57] A key concept when considering donor reactivity is the "armed-disarmed" model as coined by Fraser-Reid,[51] which explains the reactivity differences between donors bearing electron withdrawing protecting groups such as esters, and those bearing more electron donating protecting groups like ethers. Several years later, Wong and co-workers experimentally determined relative reactivity values (RRVs) for a wide range of

differently protected thioglycoside donors that amply demonstrate the armed-disarmed concept in one-pot glycosylation strategies.[57] A number of the glucose derived donors they studied are shown in **Figure 5** along with the relative reactivity value for glycosylation with methanol shown in parentheses, where a higher value denotes greater reactivity.

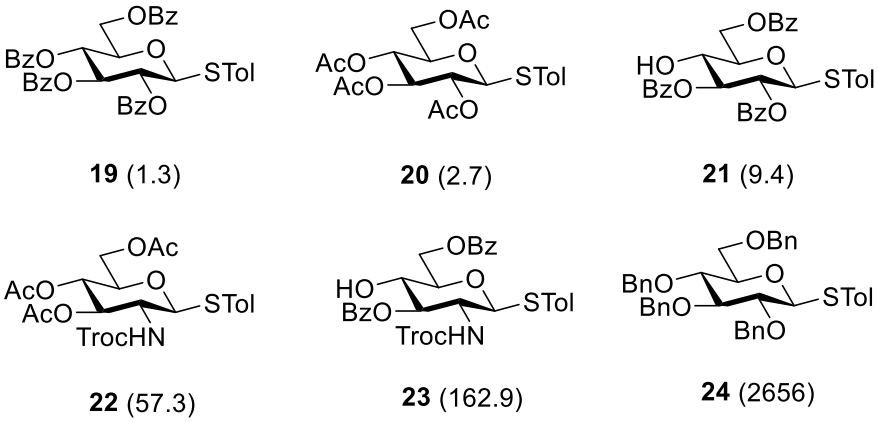


Figure 5. *Relative reactivity values for the glycosylation of some glucose derived STol donors as determined by Wong and co-workers.*[57]

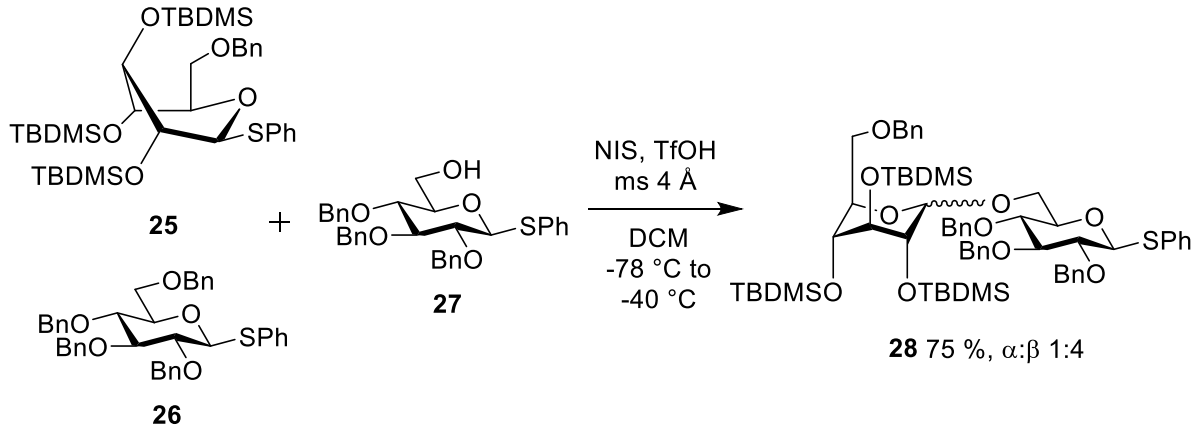
Disarmed donors bearing acetyl or benzoyl protecting groups such as **19** and **20** have the lowest reactivity values. Removing an ester functionality and replacing it with a hydroxyl group as in **21** gives a modest increase in reactivity. The presence of a less electron withdrawing carbamate protecting group at C-2 as in donors **22** and **23** gives a much greater increase in reactivity value. Finally looking to armed donor **24** bearing benzyl ether protecting groups, we see a dramatically higher reactivity value.

This armed-disarmed concept can be explained by considering that electron withdrawing groups will destabilise the oxocarbenium ion formed during a glycosylation reaction by inductively withdrawing electron density from the anomeric carbon atom and increasing the magnitude of positive charge at the atom. This effect decreases the reactivity of disarmed donors during glycosylation reactions. Conversely, electron donating groups cause electron density to accumulate at the anomeric carbon atom of the oxocarbenium ion, thus stabilising it and increasing the reactivity of armed donors. As shown in **Figure 5**, varying the identity and position of the protecting groups can change the reactivity of the donor along the armed-disarmed spectrum. By carefully selecting orthogonally protected donors along

the armed-disarmed spectrum, it is possible to facilitate sequential glycosylations and thus construct oligosaccharides in one-pot by exploiting the differing reactivities of the donors.

Building further on this concept, Bols and co-workers were able to develop a "super-armed" donor that surpasses the reactivity of other armed donors.[55] With the observation that axial polar substituents are less electron withdrawing than the same substituents in an equatorial position,[58] the researchers used bulky TBDMS protecting groups to sterically induce a conformational change in their glucose-derived donor **25**. Whilst the usual energetically preferred conformation for glucose derivatives is a chair, the researchers show that donor **25** adopts a twist-boat conformation in which the TBDMS groups are axially positioned. A competition glycosylation experiment (**Scheme 5**) in which donor **25** and armed donor **26** were put into one pot along with glycosyl acceptor **27** was performed to assess the reactivity of donor **25**. Disaccharide **28**, obtained by reaction of donor **25** with the acceptor formed in 75 % yield, confirming the higher reactivity of super-armed donor **25** over armed donor **26**. The authors attribute the reactivity to stabilisation of the intermediate oxocarbenium ion formed from donor **25** owing to the axially positioned TBDMS groups that are less electron withdrawing than equatorially orientated groups.

Demchenko and co-workers developed an alternative approach to super-armed donors.[52, 53] By combining an ester protecting group at C-2 that is capable of neighbouring group participation with electron donating benzyl ether groups at C-3, C-4 and C-6, the donor can be electronically super-armed. This was exemplified in the synthesis of super-armed donor **29** (**Figure 6**). Competition glycosylation experiments similar to those performed by Bols and co-workers demonstrated the enhanced reactivity of donor **29** over armed donors. Further work by Demchenko and Bols[50] combined both conformational and electronic super-arming strategies and incorporated them into a single donor **30** (**Figure 6**). They determined that conformational super-arming was more powerful than electronic super-arming when attempting to increase donor reactivity, since donor **30** was more reactive than donor **29**, but less so than donor **25**. However, the benzoyl group at C-2 in donor **30** allowed for complete stereocontrol during glycosylation, whereas donor **25** did not allow this level of selectivity.



Scheme 5. *Sterically super-armed donor* ***25*** *undergoes glycosylation preferentially to armed donor* ***26*** *in a competition experiment conducted by Bols and co-workers.*[55]

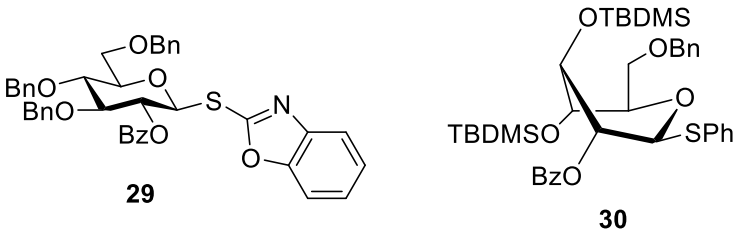


Figure 6. *Electronically super-armed donor* ***29*** *and combined conformationally and electronically super-armed donor* ***30****.*

Conformational restraints are also able to disarm donors. Commonly used acetal protecting groups, in particular 4,6-*O*-acetals, have been shown to decrease reactivity of the glycosyl donor through a combination of torsional and electronic destabilisation of the oxocarbenium ion.[59-61]

The content of this thesis concerns the investigation of novel, expedient and stereoselective glycosylation strategies that may be grouped into two categories – improved methods for stereoselective synthesis of 2-deoxyglycosides catalysed by transition metals and organocatalysts, and expediting glycosylation using chromatography-free ionic liquid supported synthesis under continuous flow conditions. Each of these research areas will be briefly evaluated through a concise survey of recent examples in the literature.

2.3. **2-Deoxyglycosides**

2-Deoxyglycosides constitute a particular class of carbohydrates that have been the challenging subject of recent synthetic attention. 2-Deoxyglycosides are monosaccharides in which no C-2 oxygen atom is present, instead two hydrogen atoms are bonded to C-2. They are frequently featured in biologically active natural products and drug compounds,[62-64] including the glycopeptide antibiotic vancomycin,[64] the enediyne antibiotic calicheamicin γ^{I}_{1}[65] and the anticancer drug aclarubicin[66] (**Figure 7**). Elucidation of the biological interactions of 2-deoxyglycoside containing compounds such as mithramycin[67] and apoptolidin[68] reveal the 2-deoxyglycoside functionality to be essential for the bioactivity of the compound. Thus, methods for the efficient construction of 2-deoxyglycosides are highly desirable.

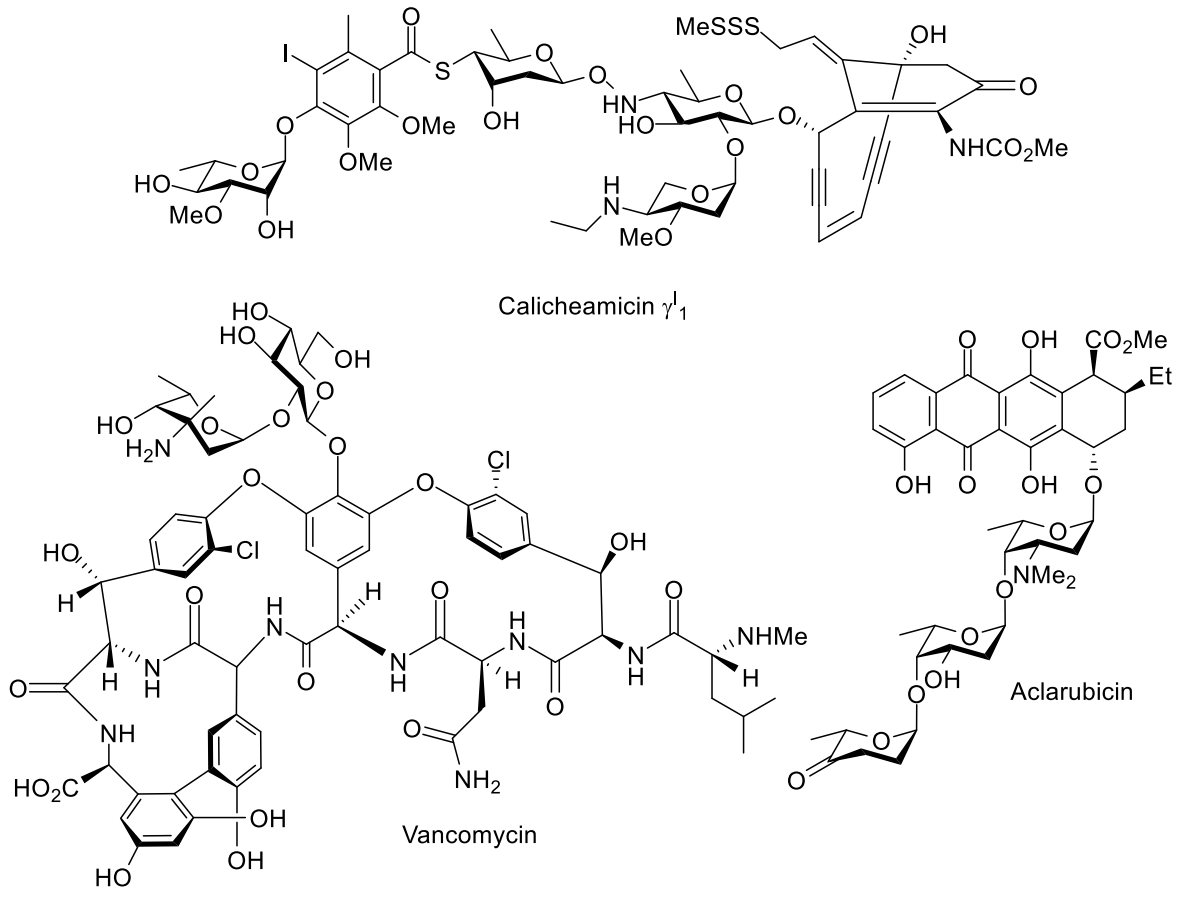


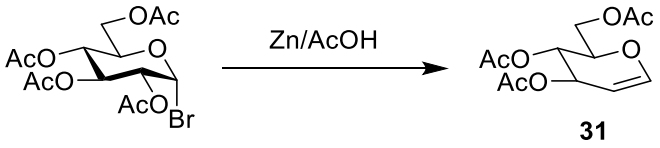
Figure 7. *Structures of vancomycin, calicheamicin γ^{I}_{1} and aclarubicin.*[8]

In the absence of a directing group at C-2, stereoselective chemical glycosylation of 2-deoxyglycosides can be challenging. Generally, α substitution is observed predominantly due

to the anomeric effect but mixtures of anomers are common. Furthermore, 2-deoxyglycosides have shown greater susceptibility to hydrolysis than their fully oxygenated counterparts, making their handling difficult.[69] This results from the increased electron density at the anomeric carbon due to the lack of an inductively electron withdrawing oxygen atom at the C-2 position, which in turn stabilises the oxocarbenium ion formed upon departure of a leaving group relative to 2-oxyglycosides. In spite of these obstacles, significant progress has been made in the field of 2-deoxyglycoside synthesis.[70-72] This section will give an introduction to glycals and their roles in 2-deoxyglycoside synthesis, followed by some of the key avenues to 2-deoxyglycosides that exist in the literature.

2.3.1. **Glycals**

Glycals are 1,2-unsaturated monosaccharide derivatives that serve as useful building blocks in carbohydrate chemistry.[73] The traditional route to glycals such as D-glucal involves treatment of a peracetylated glycosyl bromide with zinc and acetic acid.[5] In the example reaction shown in **Scheme 6** acetobromo-α-D-glucose is transformed into 3,4,6-tri-*O*-acetyl-D-glucal **31**. The harsh reaction conditions shown in **Scheme 6** are unsuitable for some substrates, however, many alternative reducing conditions are available, including Zn/Ag on graphite or lithium in liquid ammonia. In practice, preparation of glycals in a research laboratory is uncommon, since many are commercially available.



Scheme 6. *Treatment of peracetylated α-bromo-glucose with zinc/acetic acid furnishes 3,4,6-tri-O-acetyl-D-glucal* ***31****.*

Glycals can undergo a number of reactions including electrophilic addition, rearrangement and cycloadditions, with their reactivity being dictated by the enol ether functionality common to all glycals. The highly polarised C=C double bond imparts significant regioselectivity in addition reactions (**Figure 8**).

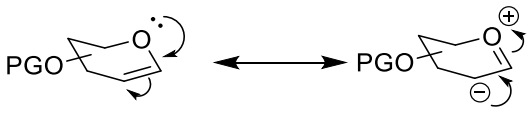
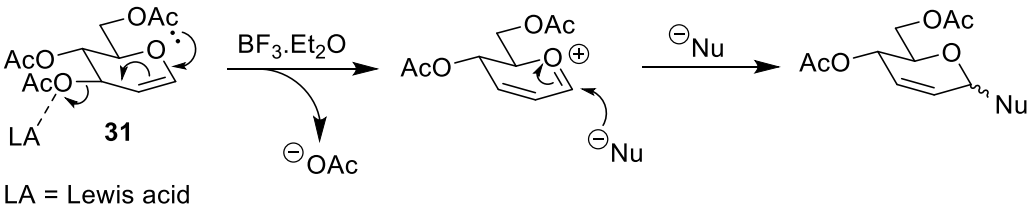


***Figure 8**. Delocalisation of charge in glycals allows highly selective addition reactions.*

Due to the partial negative charge at C-2, electrophiles such as halogens or peroxides tend to add at this position, leaving the anomeric carbon susceptible to nucleophilic attack. In the context of glycosylation, this nucleophile may be a glycosyl acceptor. This reactivity makes glycals well-suited as glycosyl donors in the synthesis of 2-deoxyglycosides.

Another well documented reaction of glycals is that of the Lewis acid catalysed allylic Ferrier rearrangement.[74, 75] In this reaction, Lewis acid catalysis induces a shift in the position of the unsaturated C=C bond from 1,2-unsaturation to 2,3-unsaturation, with concomitant loss of a leaving group at C-3 as shown in **Scheme 7** with peracetylated glucal **31**. Nucleophilic attack at the anomeric carbon by a nucleophile creates a new glycosidic bond and furnishes a hex-2-enopyranoside as product. It should be noted that the leaving group at C-3 has the potential to act as a nucleophile itself. The Ferrier rearrangement can be a versatile transformation, but it may also be an inconvenient side reaction when the intention is to prepare a 2-deoxyglycoside from a glycal donor.



***Scheme 7**. Lewis acid catalysed Ferrier rearrangement of glycals furnishes hex-2-enopyranosides.*

2.3.2. **2-Deoxyglycoside Syntheses**

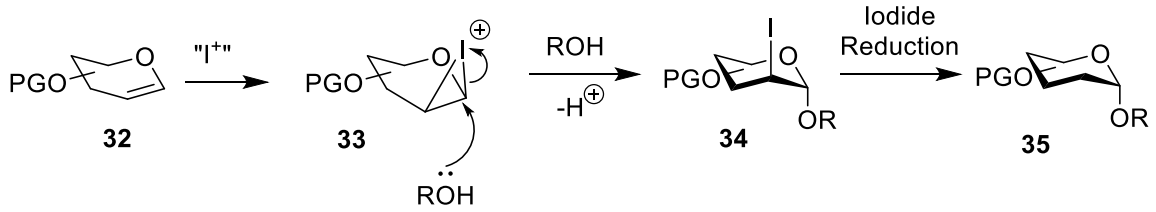
The lack of a directing group at C-2 is a major hurdle for the stereochemical control of 2-deoxyglycoside glycosylations. An approach that circumvents this problem is to use a substrate featuring a C-2 group that can engage in neighbouring group participation to install a glycosyl acceptor stereoselectively, then cleave the C-2 group to afford the 2-

deoxyglycoside once the glycosidic bond has been formed. This approach is known as an indirect synthesis. The alternative is to try to induce stereoselectivity from the stereochemical information inherent in the donor, acceptor or catalyst in a strategy known as direct synthesis.

2.3.2.1. Indirect Syntheses

One of the oldest syntheses treats a glycal with an electrophilic iodine source to give an intermediary iodonium ion that may be ring opened by a glycosyl acceptor.[76-78] **Scheme 8** shows a general preparation using this protocol. General glycal **32** is treated with an electrophilic iodine source such as NIS or iodinium-(di-*sym*-collidine) perchlorate (IDCP) to give iodonium ion **33**. The 3-membered ring will usually form on the β face of the sugar ring, with the C-1-I bond pseudoequatorial. This can be rationalised by the so-called reverse anomeric effect. This effect describes the tendency for positively charged heteroatoms bonded to the anomeric carbon to prefer an equatorial configuration for electrostatic reasons. However, the validity of the reverse anomeric effect is seriously doubted, since most substrates that show this behaviour are sterically large, and therefore the "effect" may be explained by considering steric repulsions alone.

The incoming alcohol nucleophile must attack on the α face of iodonium ion **33** due to the steric bulk of the iodine atom making attack at the β face prohibitively high in energy. Ring opening of **33** by the alcohol gives 2-deoxy-2-iodoglycoside **34**. Reductive cleavage of the iodine atom may be accomplished with either catalytic hydrogenation or radical reduction with reagents such as AIBN or tributyltin hydride to give 2-deoxy-α-glycoside **35**.

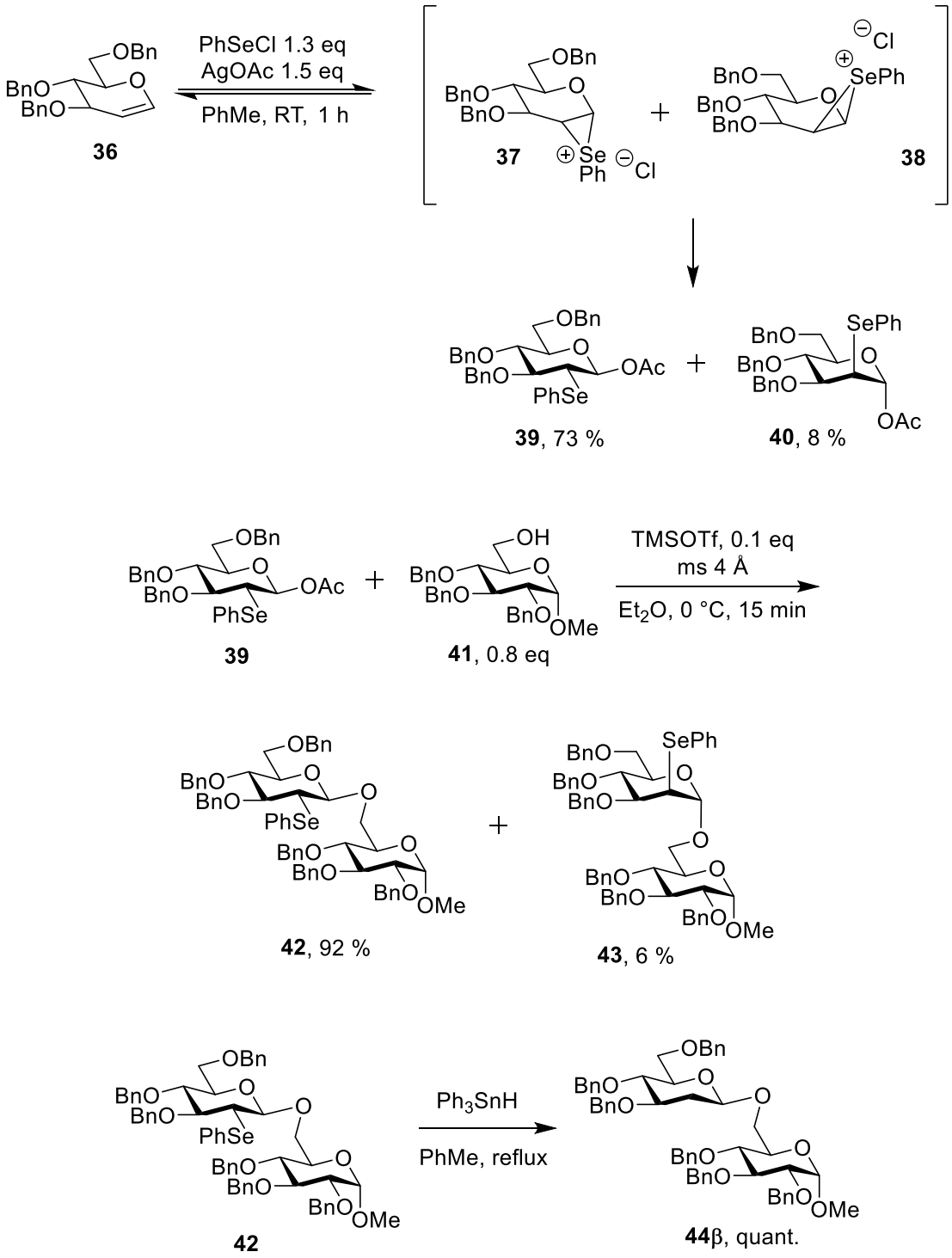


Scheme 8. *General procedure for 2-deoxyglycoside synthesis via an iodonium intermediate.*

Synthesis of 2-deoxy-β-glycosides tends to be more challenging than α anomers, since the anomeric effect must be overcome to bias formation of the β anomer. One strategy reported by Beau and co-workers[79] uses a two-step approach with the directing effect of a

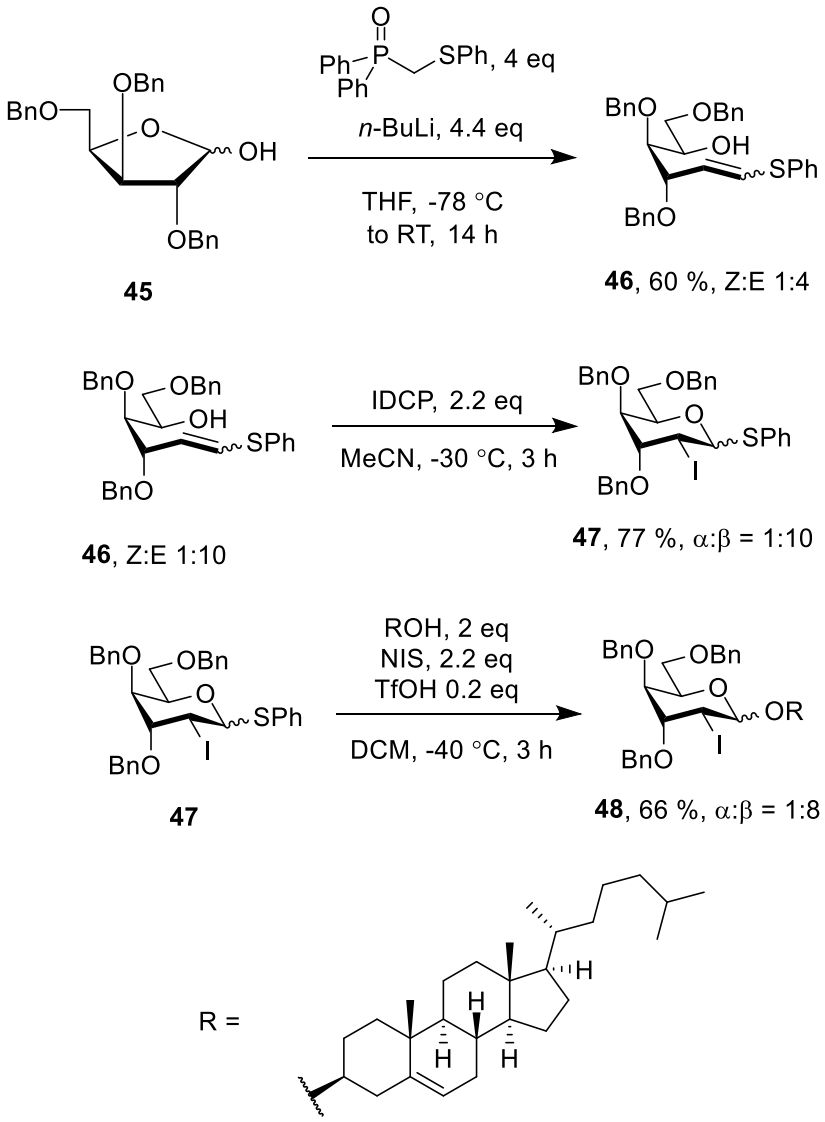
1,2-episelenonium ion dictating glycosidic bond stereochemistry. **Scheme 9** shows an example of the approach. Tri-*O*-benzyl-D-glucal **36** is treated with phenylselenyl chloride and then silver acetate is added to the reaction mixture. An equilibrium is established between glucal **36** and glycosidically pseudoaxial 1,2-episelenonium ion **37** and pseudoequatorial 1,2-episelenonium ion **38**. These ions are then trapped by the acetate anion acting as a nucleophile, attacking on the opposite face of the sugar ring to the phenylselenyl group for the same steric reasons as described above with iodonium ions. This furnishes separable 1,2-*trans* acetoxy-selenides **39** and **40**, with high selectivity for glucose derived β compound **39**. The authors note that biasing the selectivity of the reaction towards the glucose derived β compound is difficult, being sensitive to protecting groups, solvent and additives chosen.

Glycosylation of β 1,2-*trans* acetoxy-selenide **39** with glycosyl acceptor **41** using TMSOTf as promoter gives a mixture of disaccharides **42** and **43** in very high yield, with a strong preference for glucose type β anomer **42** over α anomer **43**. Interestingly, a small amount of mannose type α anomer **43** is formed, despite the glycosyl donor being purely glucose type, thus the orientation of the C-2-Se bond changes during the reaction. The researchers suggest that such a configurational change occurs through re-establishment of the equilibrium from the first reaction, with formation of glucal **36** scrambling stereochemistry. However, since the product of the reaction is biased so heavily towards β anomer **42**, it can be assumed that the amount of phenylselenyl isomerisation is small under these specific reaction conditions. Finally, treatment of **42** with triphenyltin hydride in refluxing toluene furnished 2-deoxy-β-glycoside **44β** quantitatively.



Scheme 9. *Strategy reported by Beau and co-workers for accessing 2-deoxy-β-glycosides via a 1,2-trans acetoxy-selenide intermediate.*

A more recent example of indirect 2-deoxyglycoside synthesis developed by Castillón and co-workers[80] utilises furanose precursors with a free hemiacetal group as starting materials. Wittig-Horner olefination gives alkenyl sulfanyl derivatives, then electrophilic iodinium-mediated cyclisation forms phenyl 2-deoxy-2-iodo-1-thio-hexo-glycosides. Subsequent glycosylation of the thioglycoside donors gives 2-deoxy-2-iodo glycosides in good yields and selectivities. **Scheme 10** shows an example of the multi-step strategy.

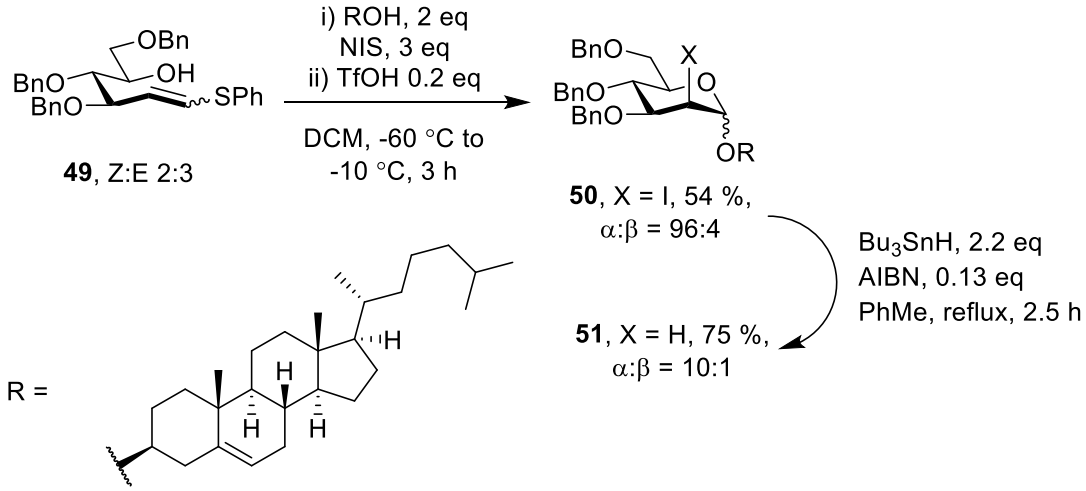


Scheme 10. *Transformation of 2,3,5-tri-O-benzyl-D-xylose* ***45*** *into 2-deoxy-2-iodo-O-glycoside* ***48*** *via alkenyl sulfanyl intermediate* ***46*** *and 2-deoxy-2-iodo-1-thio-D-gulose* ***47****.*[71, 80]

Xylose **45** undergoes a Wittig-Horner olefination using diphenyl phenylsulfanylmethyl phosphine oxide to give alkenyl sulfanyl intermediate **46** in 60 % yield as an inseparable Z:E

mixture. Treatment of **46** with IDCP forms an iodonium ion and induces ring closure to give gulose **47** in 77 % yield and good β selectivity. Several examples of different 2-deoxy-2-iodo-1-thioglycosides were synthesised. In each case, a 6-endo cyclisation occurred with the C-2-I bond exhibiting a *cis* relationship with the C-3 substituent almost exclusively. Glycosylation of thioglycoside **47** with cholesterol as the glycosyl acceptor using NIS/TfOH as promoters furnished *O*-glycoside **48** in 66 % yield and 1:8 α:β selectivity. Glycosylation selectivities using cholesterol as the glycosyl acceptor ranged from 1:8 to 1:37 in favour of a 1,2-*trans* relationship between the C-1-O bond and the C-2-I bond.

The Castillón group went on to develop the system into a one-pot electrophile-induced cyclisation-glycosylation, shown in **Scheme 11**.[81] Alkenyl sulfanyl species **49** was treated with an excess of *N*-iodosuccinimide to firstly induce ring closure, then subsequent addition of triflic acid activated the thiophenyl leaving group. Departure of the leaving group and addition of glycosyl acceptor cholesterol gives 2-deoxy-2-iodoglycoside **50** in 54 % yield and very high α selectivity in accordance with the previously observed propensity for 1,2-*trans* glycosylation in this system. Radical mediated dehalogenation furnished 2-deoxyglycoside **51** in 75 % yield.



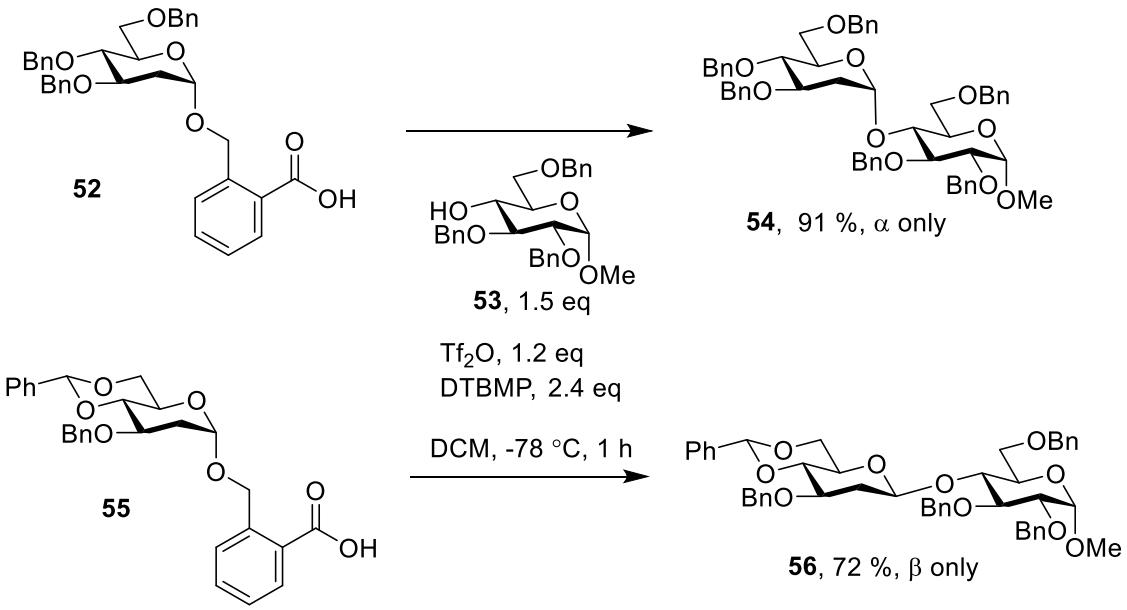
Scheme 11. *One-pot cyclisation-glycosylation procedure leading to 2-deoxyglycoside* ***51****.*[81]

Whilst indirect 2-deoxyglycoside preparations are able to impart very good selectivity in both 1,2-*trans* and 1,2-*cis* fashion through different strategies, they are inherently inefficient. The installation and removal of the directing group will require at least two

additional steps in any glycosylation strategy, which will almost certainly lead to a reduction in yield and a lengthier preparation. An alternative route that has received considerable attention in recent years is that of direct synthesis.

2.3.2.2. Direct Syntheses

A recent route to 2-deoxyglycosides reported by Kim and co-workers uses (2'-carboxyl)benzyl glycosides as glycosyl donors in a direct synthesis approach.[82] Intriguingly, small changes in the donor protecting groups lead to complete reversal of stereoselectivity. **Scheme 12** shows examples of this stereocontrol.

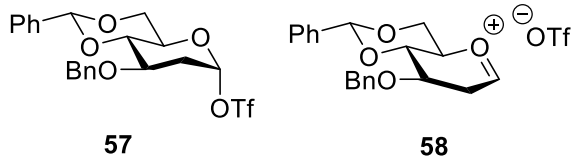


Scheme 12. *Glycosylation of differently protected (2'-carboxyl)benzyl glycosides gave 2-deoxydisaccharides with opposite stereochemical outcome.*[71, 82]

Glucose derived glycosyl donor **52** made from tri-*O*-benzyl-D-glucal **36**, can be activated with triflic anhydride and the hindered base DTBMP to undergo glycosylation with secondary glycosyl acceptor **53**, giving disaccharide **54** in excellent yield and total α selectivity. Other secondary glycosyl acceptors also showed preference for formation of the α anomer, with α:β ratios from 9.4:1 to complete α selectivity. In contrast, when the C-4 and C-6 benzyl protecting groups on the glycosyl donor were replaced with a benzylidene acetal group as in donor **55**, the same reaction conditions gave disaccharide **56** in good yield and complete β selectivity. Other secondary acceptors showed α:β selectivity from 1:8 to complete β

selectivity when donor **55** was used. For both donors **52** and **55**, little or no stereoselectivity was observed when primary glycosyl acceptors were used, with α:β ratios close to 1:1.

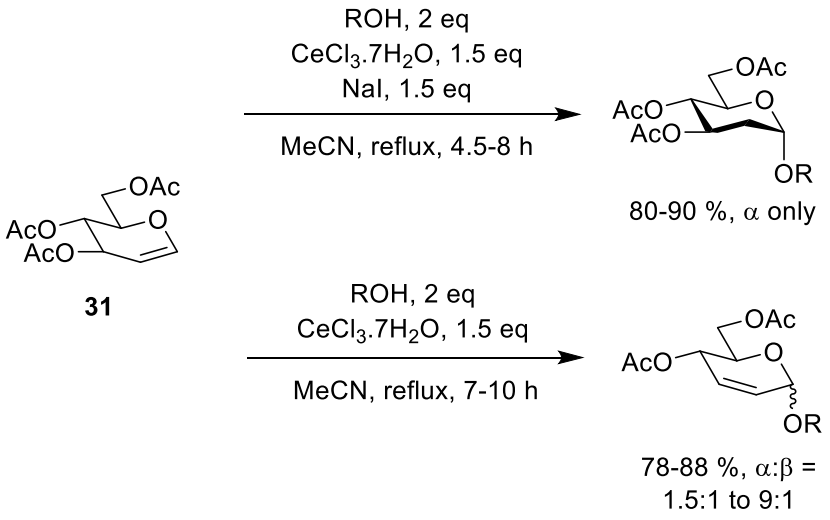
The Kim group rationalised the observed selectivities by suggesting that reactions between donor **55** and secondary glycosyl acceptors proceeded via intermediate **57** in an S_N2 type mechanism to give inversion of stereochemistry, whilst in the case of primary acceptors the reaction proceeded through oxocarbenium ion **58** in an S_N1 type manner, thus imparting little selectivity (**Figure 9**). However, no mechanistic studies were performed and intermediate **57** was not detected.



57 **58**

Figure 9. *Proposed α-glycosyl triflate intermediate **57** and oxocarbenium ion **58**.*[71, 82]

Another example of a direct 2-deoxyglycoside synthesis that makes use of glycals as glycosyl donors was reported by Satyanarayana and co-workers.[83] The lanthanide cerium salt $CeCl_3.7H_2O$ may be used in combination with sodium iodide to effect glycosylation of peracetylated glycals with a range of aliphatic, aromatic, olefinic and alkynylic primary alcohols. **Scheme 13** shows a general scheme of the reactions performed. Acetylated glucal **31** was treated with $CeCl_3.7H_2O$ and sodium iodide and an alcoholic glycosyl acceptor to give selectively α 2-deoxyglycosides in very good yields. The reaction worked well even when water was not strictly excluded from the reaction vessel as is normally necessary for glycosylation reactions. The authors note that treatment of glucals with the cerium(III) salt in the absence of sodium iodide gave the 2,3-unsaturated Ferrier rearrangement product in very good yield, but poor to moderate selectivity. The researchers suggest that glycal activation by hydroiodic acid formed *in situ* was responsible for the observed reactivity, giving 2-deoxyglycoside products. Unfortunately, the authors did not make any suggestions as to the tolerance of the method for saccharide derived glycosyl acceptors, secondary alcohols or for protecting groups other than the acetyl group.



Scheme 13. *Transformation of peracetylated D-glucal* ***31*** *into 2-deoxyglycosides and Ferrier rearrangement products using* Ce^{III} *chloride.*

These examples of direct 2-deoxyglycoside synthesis and many more that are beyond the scope of this introduction demonstrate that efficient, high yielding and selective direct preparations of 2-deoxyglycosides are possible if the donor, acceptor and catalyst/promoter reagents are carefully selected. Ideally, a highly tolerant and efficacious methodology can be developed for 2-deoxyglycoside synthesis, using synthetically versatile glycals as glycosyl donors.

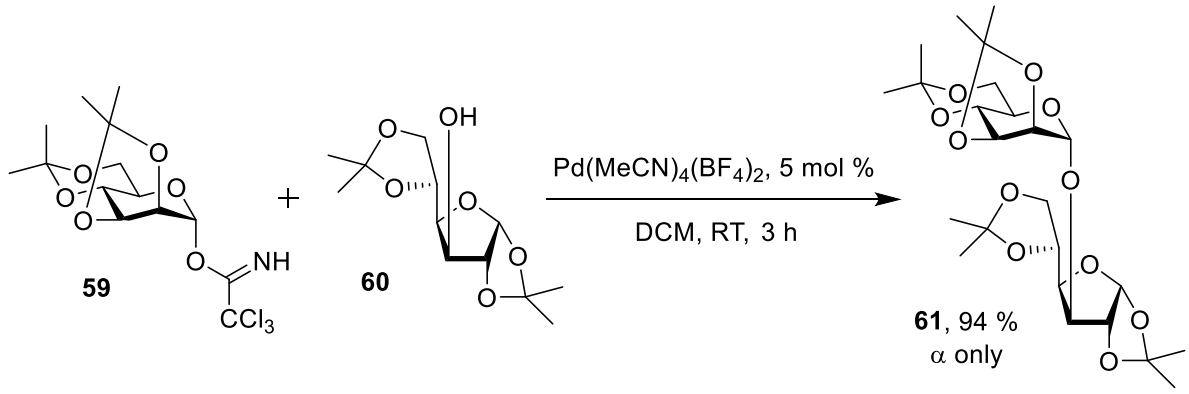
2.4. **Glycosylation by Transition Metal Catalysis**

Catalysis is the widespread chemical process by which reaction rate is increased using a catalyst, a substance that accelerates the reaction without being consumed itself. This occurs because the catalyst offers a reaction pathway that requires lower activation energy than the uncatalysed pathway. Generally, sub-stoichiometric quantities of catalyst are used, since the catalyst should be regenerated after each reaction cycle and thus acts repeatedly. Transition metals are used extensively as catalysts, with transition metal catalysis having become an incredibly varied and valuable field. Transition metals as both homogeneous and heterogeneous catalysts are essential for large scale industrial chemical production as well as almost all research lab scale branches of organic chemistry. Of particular note is the explosion

of research that has gone into palladium mediated C-C and C-heteroatom bond formation, for example the Heck reaction[84] and the Buchwald-Hartwig amination/etherification.[85, 86]

Metal catalysts are able to exploit reactivity in organic chemicals that is inaccessible by traditional organic synthesis, offering the opportunity for rapid, highly selective transformations. This reactivity is primarily due to the partial occupation of d orbitals exhibited by transition metal catalysts. The d orbitals possess differing symmetry to that of the s and p orbitals occupied by carbon-based organics, allowing orbital interaction of organics and metal atoms that leads to synthetically useful bond breaking and forming reactions. Moreover, due to the developing fields of green chemistry and sustainability,[87] science and chemistry in particular must respond to the demand for more efficient, atom economic, environmentally innocuous and economically viable processes. Transition metal catalysts represent a path to this goal, by reducing wasteful volumes of reagent, solvent and catalyst, improving safety in the laboratory and in industrial plants whilst motivating innovation in chemistry. Transition metal catalysis has seen extensive exploitation for glycoside synthesis in recent years.[28, 33]

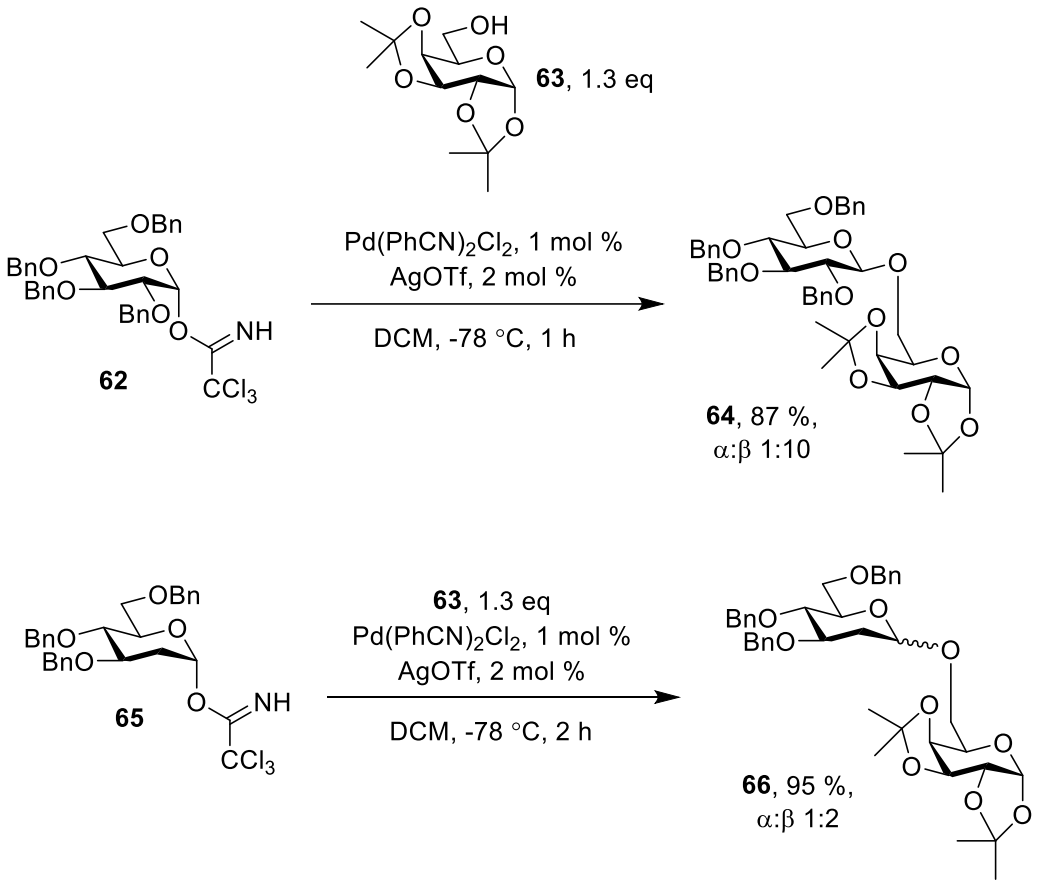
Glycosyl trichloroacetimidates are perhaps one of the most widely used glycosyl donors, owing to their ease of preparation and synthetic versatility.[88] Hence, Nguyen and co-workers opted to apply palladium(II) catalysis to the glycosylation of trichloroacetimidate donors.[89] By employing 5 mol % of cationic $Pd(MeCN)_4(BF_4)_2$ as the catalyst, various glycosylations were performed giving products in high yields and excellent stereoselectivities. For instance, glycosylation of mannose donor **59** with secondary acceptor **60** furnished disaccharide **61** in 94 % yield and complete α selectivity (**Scheme 14**).



Scheme 14. *Glycosylation of mannosyl trichloroacetimidate donor **59** with acceptor **60** using $Pd(MeCN)_4(BF_4)_2$ as catalyst.*[89]

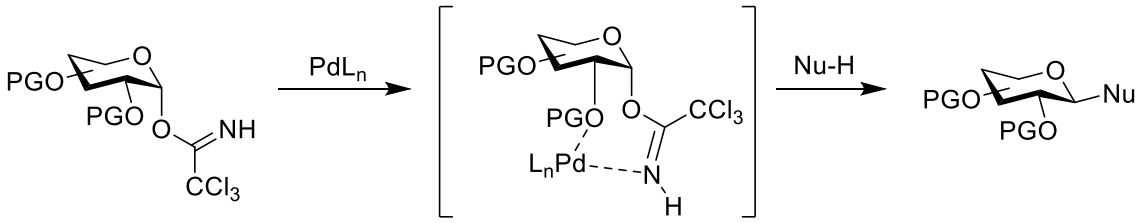
The non-coordinating nature of the tetrafluoroborate counterions was shown to be integral to catalytic activity, since the use of complex $Pd(PhCN)_2Cl_2$ as an alternative catalyst gave only very low (<5 %) yields. Mechanistically, the palladium complex is proposed to act as a Lewis acid, encouraging departure of the trichloroacetimidate group to give an oxocarbenium ion that may be attacked by a nucleophile. The reaction worked well for glycosyl donors that possess an axial C-2 group, or with donors capable of anchimeric assistance, however, stereoselectivity deteriorated when using a donor with non-participating functionality.

In pursuance of a solution to the loss of selectivity, a second-generation catalyst was developed by the Nguyen group that utilised very weakly coordinating triflate counterions, thereby enhancing the cationic character of the metal centre and increasing catalyst activity. $Pd(PhCN)_2(OTf)_2$, generated *in situ* from $Pd(PhCN)_2Cl_2$ and AgOTf, proved to be a highly active catalyst for the β selective glycosylation of glycosyl trichloroacetimidates bearing various different ether-type protecting groups.[90] The protocol also tolerates a range of glycosyl acceptors. **Scheme 15** shows how perbenzylated glucosyl donor **62** can be smoothly glycosylated with galactose derived acceptor **63** in high yield and β selectivity to give disaccharide **64**. The authors also demonstrate that 2-deoxyglycosides can be prepared using this method, as shown in the synthesis of **66** directly from donor **65**.



Scheme 15. *Glycosylation of glycosyl trichloroacetimidate donors using catalyst $Pd(PhCN)_2(OTf)_2$ generated in situ furnished disaccharides in high yields and β selectivities.*

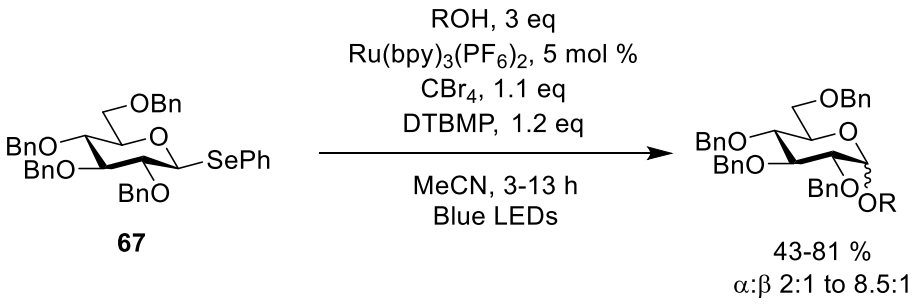
Whilst the yield for the preparation of **66** is very high, the selectivity is much lower than for other fully oxygenated donors. The authors proposed that the high β selectivity seen when fully oxygenated donors are used stems from the palladium atom coordinating the imidate N atom and the C-2-O atom simultaneously to form a transient 7-membered ring, which directs nucleophilic attack to the β face (**Scheme 16**). Accordingly, in 2-deoxy donor **65**, no C-2-O atom is present to coordinate the Pd and therefore β selectivity deteriorates dramatically. The synthesis of oligosaccharides is also amenable to this method, as exemplified in the synthesis of a trisaccharide. The work was subsequently extended to permit glycosylation using aryl alcohols as glycosyl acceptors to give β-*O*-aryl glycosides selectively.[91]



Scheme 16. *Mechanistic pathway proposed by Nguyen and co-workers for the β selective Pd^{II} catalysed glycosylation.*[90]

The use of metal complexes that can participate in single electron transfer redox reactions under the influence of photochemical excitation is emerging as a powerful tool for catalysis.[92] This reactivity was exploited by Ragains and co-workers for the α selective glycosylation of selenoglycosides.[93] By using a catalytic quantity of $Ru(bpy)_3(PF_6)_2$ in combination with tetrabromomethane as an electron acceptor, a base and irradiation by blue LED light, selenoglycosides were glycosylated in good yields and reasonable selectivity. Four examples of glycosylation using selenoglucoside **67** and several alcohol acceptors are reported, with α:β selectivities up to 8.5:1 and yields up to 81 % (**Scheme 17**). It should also be noted that the reaction also worked with organocatalytic diphenylselenide in place of the ruthenium complex.

The proposed catalytic cycle for the reaction is outlined in **Figure 10**. The cycle begins with irradiation of the $Ru(bpy)_3^{2+}$ complex with blue light, causing electron excitation. Tetrabromomethane is then able to abstract an electron from the excited complex to give $Ru(bpy)_3^{3+}$, which may oxidise the selenoglycoside donor **67** to give radical cation **68** and regenerate $Ru(bpy)_3^{2+}$. Radical cation **68** then rapidly fragments to produce an oxocarbenium ion that is susceptible to nucleophilic attack by the glycosyl acceptor, furnishing the product glycoside.



Scheme 17. *Visible light mediated glycosylation using a ruthenium complex as a single electron transfer reagent.*[93]

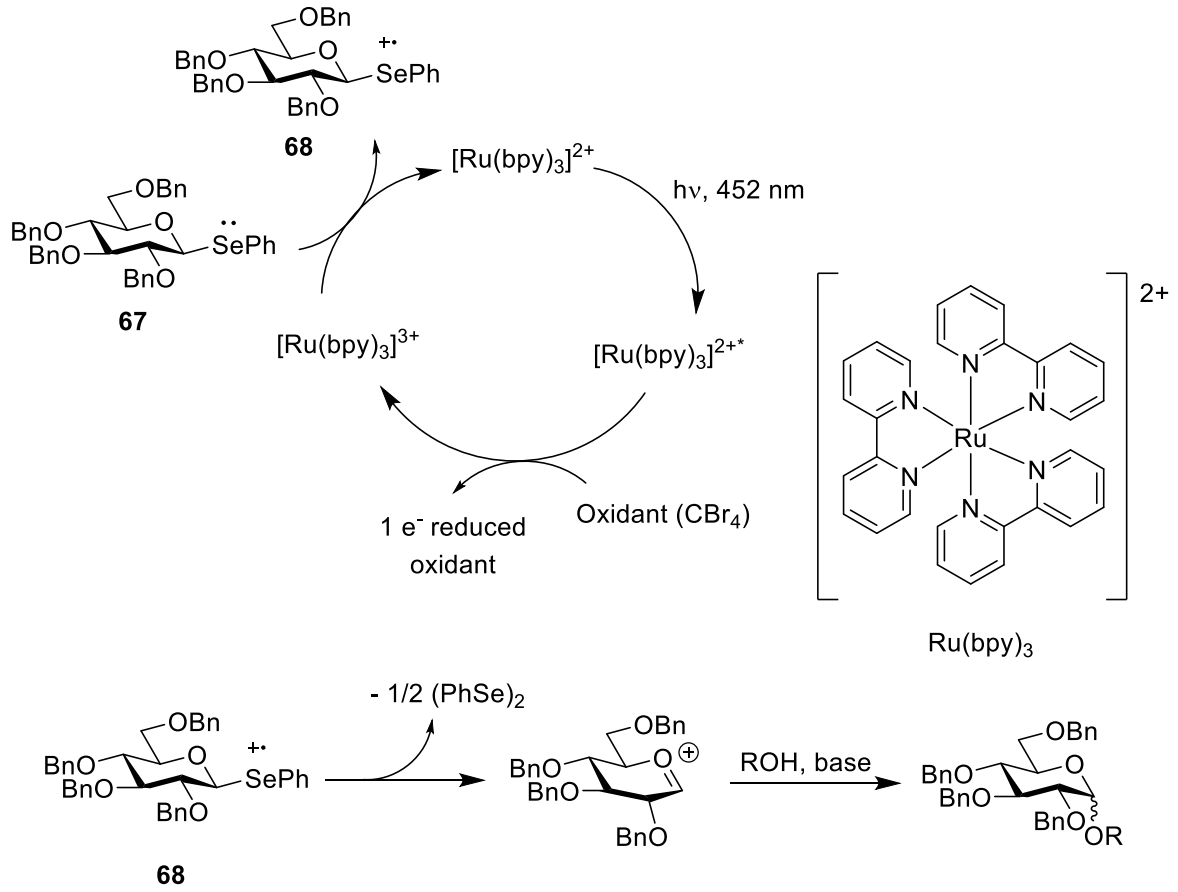
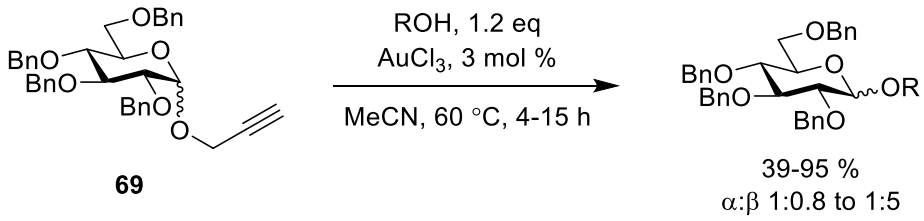


Figure 10. *Proposed catalytic cycle for the photoredox catalysed glycosylation of selenoglycosides.*

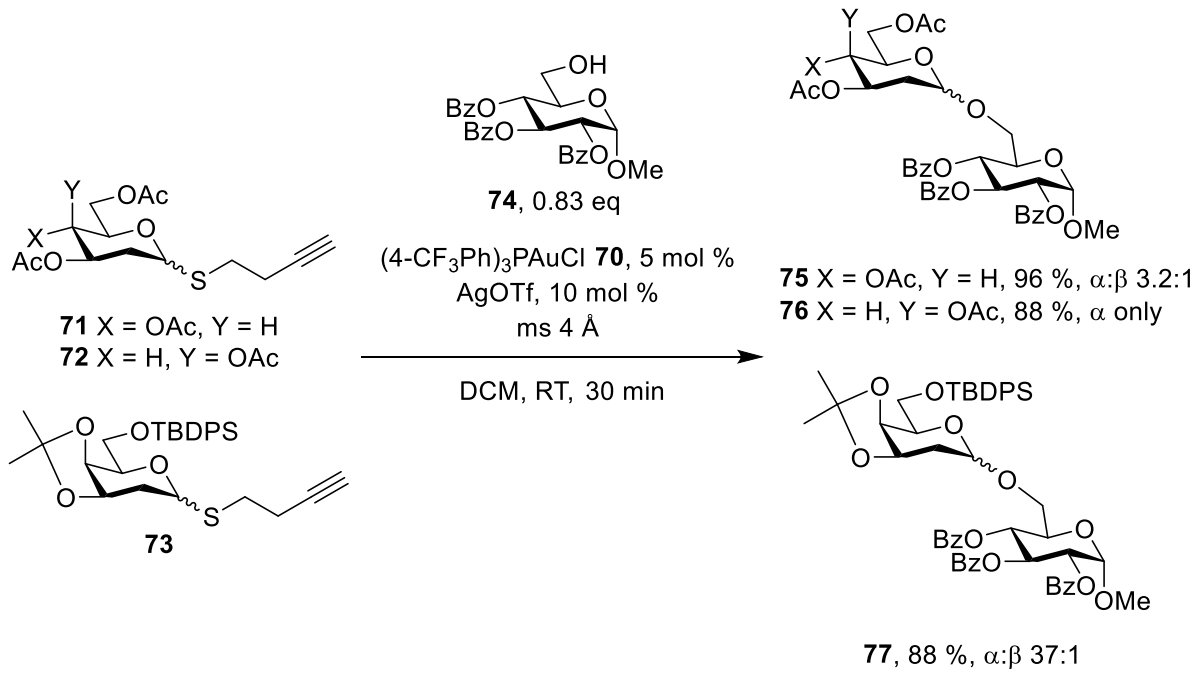
An ideal requirement for successful and versatile glycosylation strategies is that of chemical orthogonality. Ideally, one anomeric latent leaving group will be activated under specific conditions which ought not to interfere with other chemical functionalities present in the reaction vessel, whilst another leaving group can be activated under alternative conditions, enabling facile oligosaccharide synthesis. Hence, the search for anomeric leaving groups that remain stable under most conditions, only becoming reactive when convenient, is a tantalising prospect. Building upon the notable alkynophilicity exhibited by gold catalysts,[94-96] Kashyap and co-workers sought to develop propargyl groups as anomeric leaving groups that could be activated through gold catalysis.[97] Using $AuCl_3$ as a catalyst, propargyl glucosyl donor **69** was shown to be smoothly glycosylated with six examples of alcohols, including aromatic, alkenyl and saccharide derived acceptors (**Scheme 18**). Yields were fair to excellent, however α:β stereoselectivities were generally poor. Two further glycosyl donors bearing benzyl protecting groups were amenable to the reaction conditions, however, electron withdrawing ester protecting groups were not tolerated, suggesting that the reaction works best with armed donors, whilst disarmed donors lack the reactivity to be activated by the gold catalyst. The authors went on to suggest a plausible mechanism in which gold activation of the alkyne moiety causes departure of the propargyl leaving group, but did not offer experimental information in support of it.



Scheme 18. *Au^{III} catalysed glycosylation of glycosyl donor **69** bearing a propargyl leaving group at the anomeric position.*

Zhu and co-workers further extended the use of alkynyl glycosyl donor activation with gold catalysis by applying the strategy to the synthesis of 2-deoxy- and 2,6-dideoxyglycosides.[98] A series of 2-deoxy glycosyl donors bearing an *S*-but-3-ynyl group at the anomeric position were prepared. Treatment with catalytic gold(I) species **70**, featuring an appropriate phosphine ligand, and silver triflate afforded products in short reaction times

under very mild conditions (**Scheme 19**). Glucosyl donor **71** gave product **75** in very high yield in the reaction with acceptor **74**, however, the selectivity was reasonably low. In contrast galactose derived donors **72** and **73** afford products **76** and **77** in the reaction with acceptor **74** with high preference for the α anomer.



***Scheme 19**. Gold(I) catalysed glycosylation of 2-deoxy-S-but-3-ynyl thioglycoside donors using glycosyl acceptor **74** afforded 2-deoxy glycosides in high yields and α selectivities.*

The authors detected 2,3-dihydrothiophene **82** as a side product of the reaction and proposed the mechanism shown in **Figure 11**. Gold catalyst **78** coordinates with the triple bond of donor **71** to form complex **79**. 5-endo-dig cyclisation forms species **80**, which then fragments to make gold complex **81** and an oxocarbenium ion. The glycosyl acceptor alcohol traps the oxocarbenium ion affording the product, whilst the liberated proton permits proto-deauration of complex **81** to create 2,3-dihydrothiophene **82** and regenerate gold catalyst **78**.

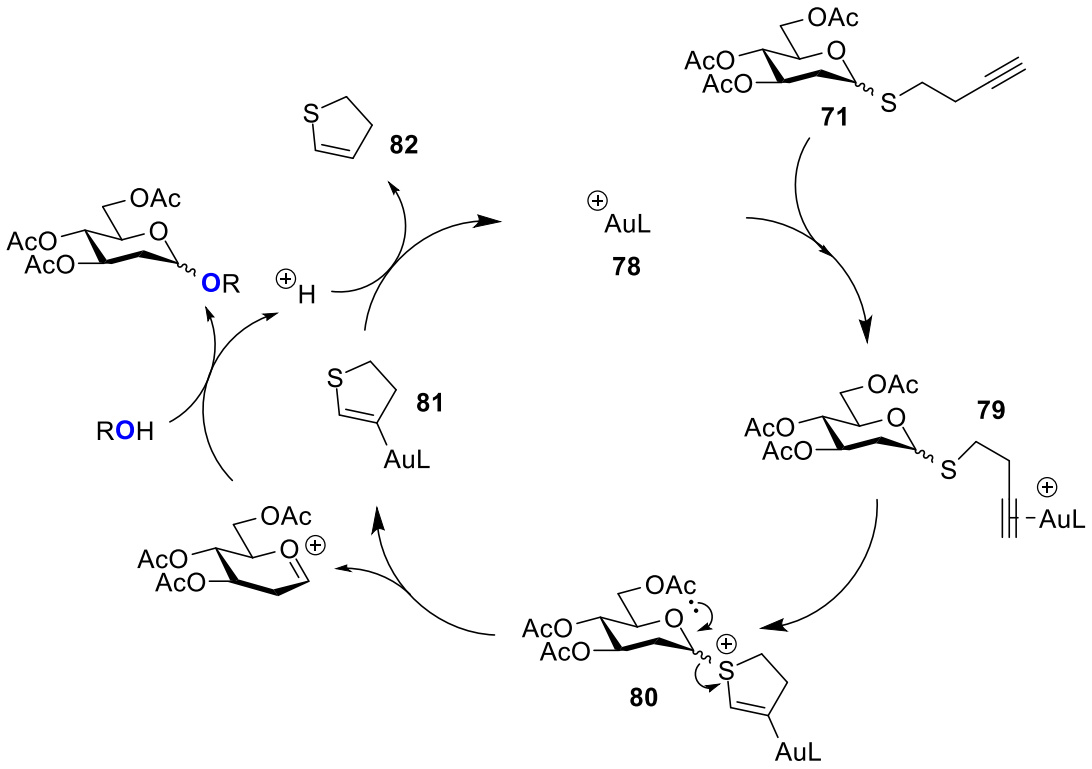
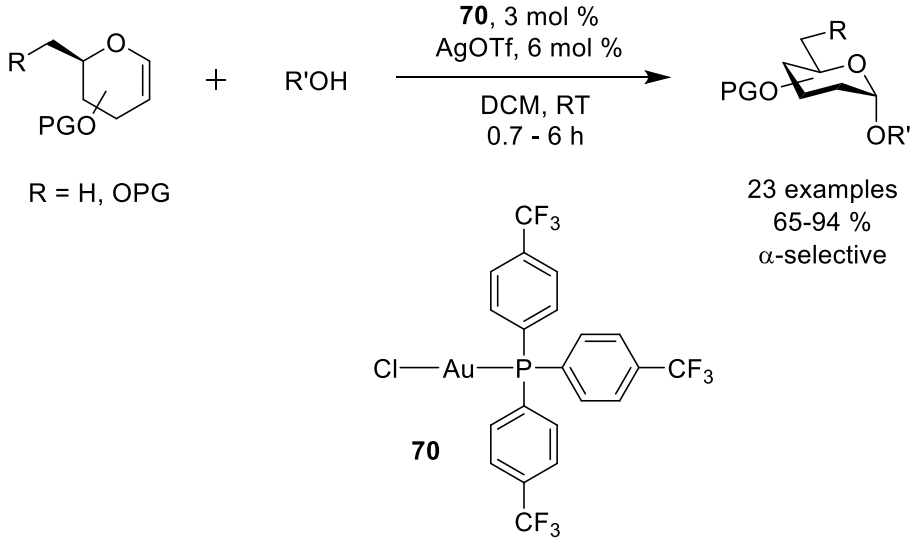


Figure 11. *Proposed catalytic cycle for the gold(I) catalysed synthesis of 2-deoxyglycosides reported by Zhu and co-workers.*[98]

Building further upon gold catalysed glycosylations, Galan and co-workers also used gold catalyst **70**, but instead of activating an alkyne moiety, the enol ether group of the glycal donor was activated (**Scheme 20**).[99] This glycosylation protocol utilised galactal, glucal and rhamnal type donors in combination with a wide variety of glycosyl acceptors to synthesise 2-deoxyglycosides with excellent α selectivities of generally >30:1 α:β. Reactions reached completion quickly, usually requiring just 35 minutes at room temperature for complete conversion. Moreover, the method can be used to prepare oligosaccharides, as evidenced by the synthesis of a tetrasaccharide in 18 % overall yield over five steps.



Scheme 20. *Gold(I) catalysed glycosylation of glycals to produce 2-deoxyglycosides with excellent α-selectivity, as reported by Galan and co-workers.*[99]

The field of transition metal catalysed glycosylations is already large and will undoubtedly continue to grow. For an exhaustive evaluation of transition metal catalysed glycosylations to date, two recent reviews cover the topic in depth.[28, 33] The use of transition metals allows much better stereochemical control of glycosylation reactions than traditional methods through formation of transient metal complexes that may direct attack of the glycosyl acceptor. Furthermore, the small amount of metals required for efficient glycosylation minimises waste whilst allowing mild conditions. It should also be noted that transition metal catalysts facilitate the use of novel glycosyl donors that can be chemoselectively activated, amplifying the scope for orthogonal glycosylation strategies. However, a complementary approach to glycosylation chemistry with its own distinct advantages uses organocatalysts.

2.5. **Glycosylation by Organocatalysis**

A viable alternative to transition metals is organocatalysis, which relies on organic molecules as catalysts. Difficult synthetic transformations in many areas of chemistry have been accomplished using a rationally selected small organic molecule to catalyse the reaction. Investigation into this field has led to efficient routes to target molecules, with high chemo-, regio- and enantioselectivity.[100-103] Furthermore, organocatalysis provides a green pathway

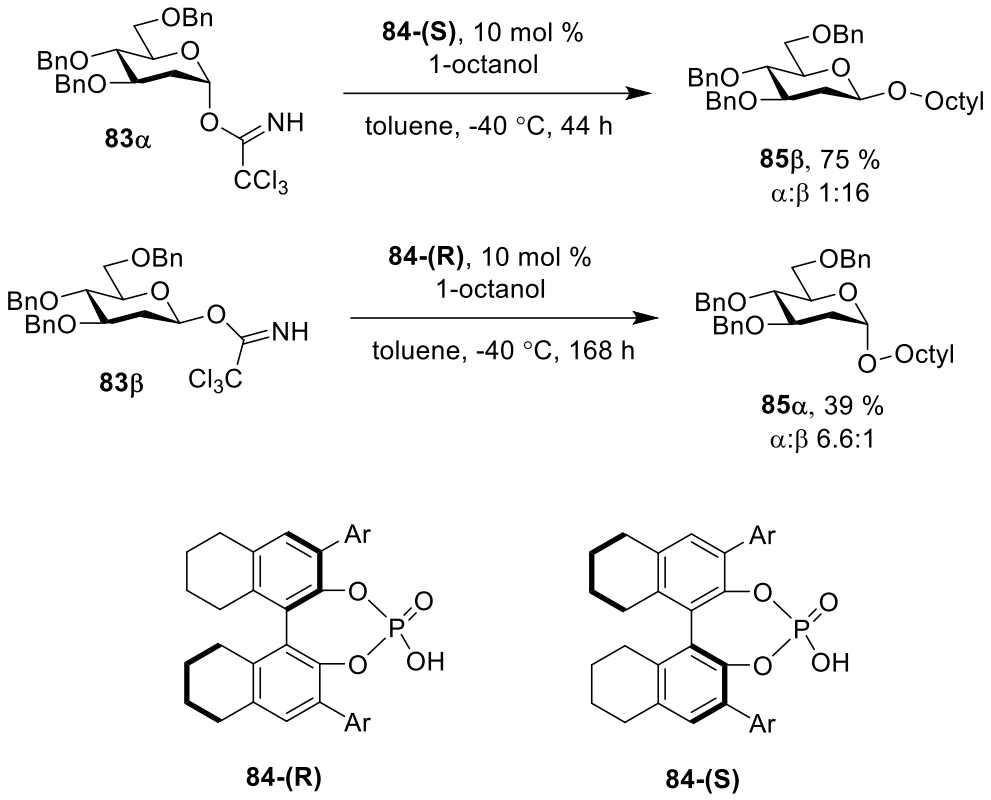
to desired compounds without requiring the use of transition metal catalysts, thus circumventing the potential expense, toxicity and environmental harmfulness associated with many transition metals.[87]

Organocatalysts, particularly in the case of Brønsted acid or hydrogen bonding catalysts, possess several distinct advantages over more traditional Lewis acidic metal catalysts. Organic transformations including glycosylations have been catalysed using Lewis acidic metals for decades. Commonly, promoters including salts of tin, silver and mercury are used for traditional glycosylation. However, due to the enthalpic tendency towards coordination of electron deficient metals by heteroatoms, metal catalysts are frequently sensitive to moisture and air. Furthermore, the metal catalyst may be poisoned through strong binding to the glycosylation product. Conversely, organocatalysts often bind more weakly to the reactants and products thereby avoiding these issues, although this may also result in reduced catalytic turnover frequency.[104]

On the other hand, strong Brønsted acids such as mineral acids may be used to catalyse glycosylations. Yet they often suffer from poor chemoselectivity due to indiscriminate interaction with functional groups throughout the reactant molecule, leading to unwanted side products. Organic Brønsted acids are generally much weaker than standard strong Brønsted acids, permitting superior chemoselectivity, tolerance of diverse functional groups and often greater stereocontrol during glycosylations.

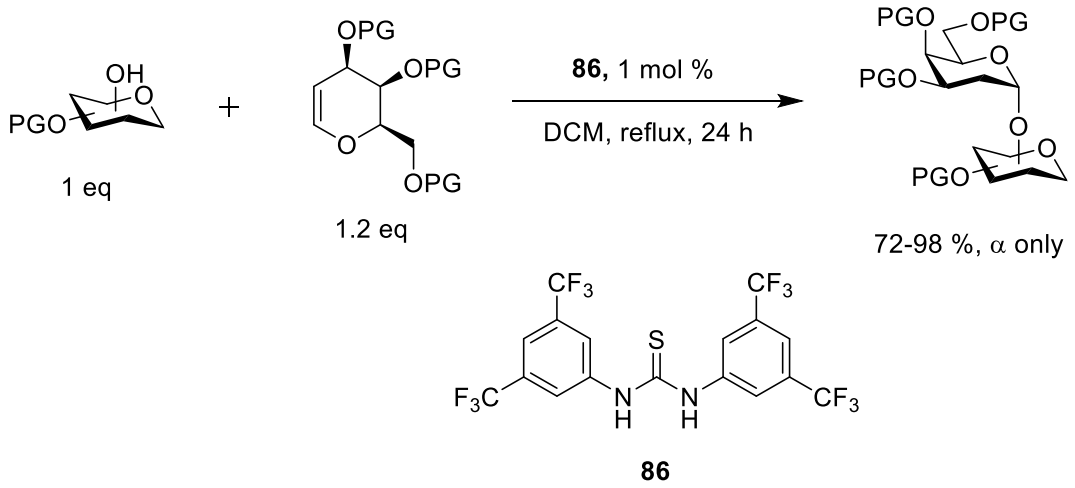
Another factor that contributes to the attractiveness of organocatalysts is their modifiability. Since they are often easily synthesised from simple starting materials, the steric and electronic nature of the catalyst can be tuned to the requirements of the reaction. Moreover, chiral motifs can be incorporated into the organocatalyst structure, thus imparting stereochemical information during the catalytic reaction and biasing the stereochemical outcome of the reaction. The application of organocatalysis to the glycosylation reaction has the potential to minimise waste, provide an orthogonal glycosylation strategy to most existing methods whilst remaining tolerant to protecting groups and allowing rapid access to densely functionalised oligosaccharides under mild conditions. This part of the introduction will outline some recent examples of organocatalytic glycosylation strategies.[105]

In 2014, Bennett and co-workers reported an organocatalytic BINOL derived phosphoric acid that was able to selectively catalyse the formation of different 2-deoxyglycoside anomers dependent upon the matched/mismatched chiral relationship between the organocatalyst and the glycosyl donor anomeric configuration.[106] For their study, perbenzylated 2-deoxy trichloroacetimidate glycosyl donors **83α** and **83β** were chosen. It was discovered that by using sterically bulky chiral Brønsted acid catalysts **84-(S)** and **84-(R)** for the glycosylations with 1-octanol as glycosyl acceptor, both anomers of the product, **85α** and **85β**, could be preferentially synthesised. **Scheme 21** shows that treatment of α donor **23α** with the (S) enantiomer of the Brønsted acid catalyst **84-(S)** affords synthetically challenging β 2-deoxyglycoside **85β** in excellent yield and selectivity, whilst using the (R) enantiomer of the catalyst (**84-(R)**) with the same donor deteriorated selectivity and lengthened reaction time. Conversely, the glycosylation of **83β** using **84-(R)** gave the α anomer of the product **85α** preferentially. However, the reactions had to be run at low temperature owing to the instability of the glycosyl donors, leading to exceptionally lengthy reaction times. When a saccharide derived glycosyl acceptor was used in place of 1-octanol in the glycosylation of **83α**, the β selectivity was largely maintained (α:β 1:14), however, the yield dropped to 15 %. Nevertheless, the study amply demonstrated the importance of matching the chirality of the organocatalyst with the glycosyl donor in order to maximise stereocontrol during the glycosylation.



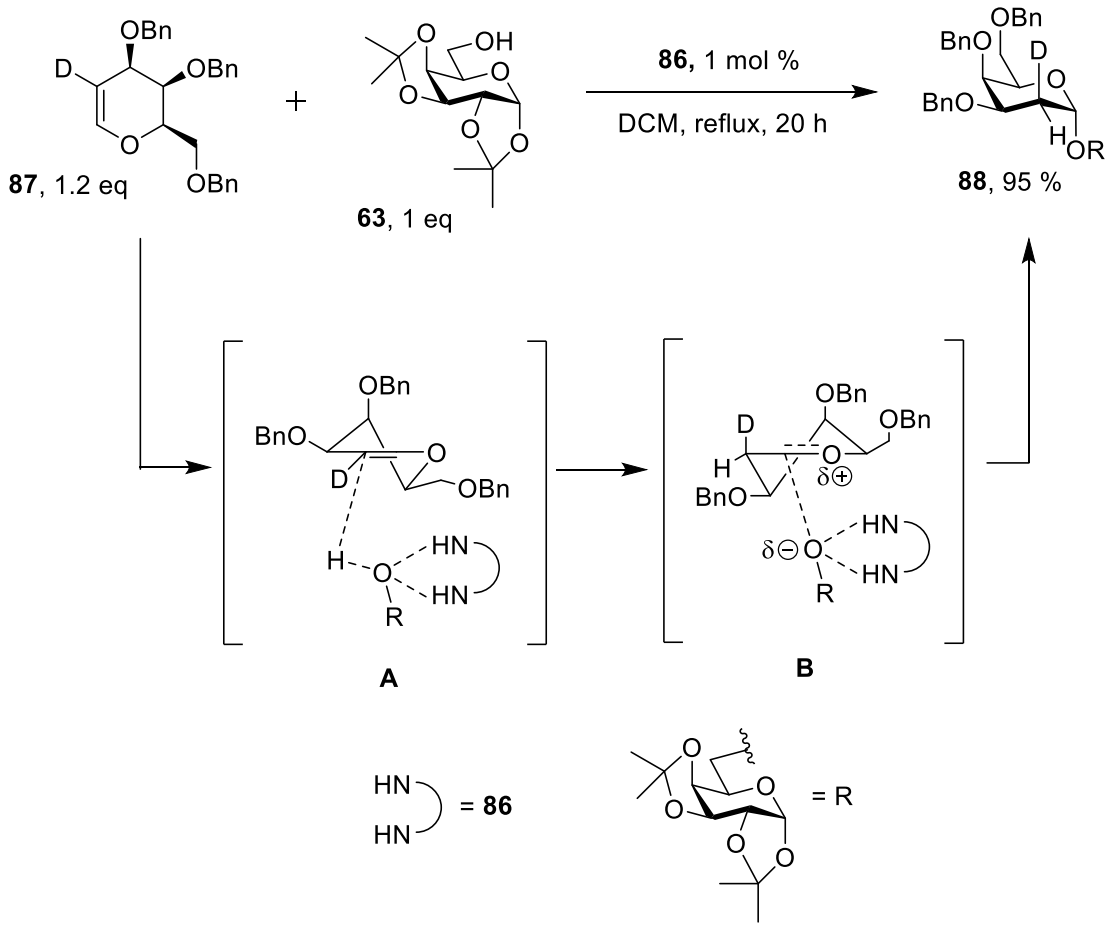
Scheme 21. *Glycosylation using the matched/mismatched catalyst/substrate methodology developed by Bennett and co-workers gives 2-deoxyglycosides stereoselectively.*[106]

Inspired by reports that electron deficient thiourea **86** efficiently catalyses the tetrahydropyranylation of alcohols,[107] the Galan group set out to apply this methodology to suitably protected glycals as a novel route to access 2-deoxyglycosides.[108] Using thiourea **86** at low catalyst loadings, a wide variety of galactals bearing diverse protecting groups were glycosylated over 24 hours at reflux in DCM (**Scheme 22**). The yields were excellent and complete α-selectivity was achieved. The method was amenable to both primary and secondary glycosyl acceptor alcohols. However, the reaction did not tolerate glucal donors well, since reactions using glucals were slower, less stereoselective and lead to more side products.[109]



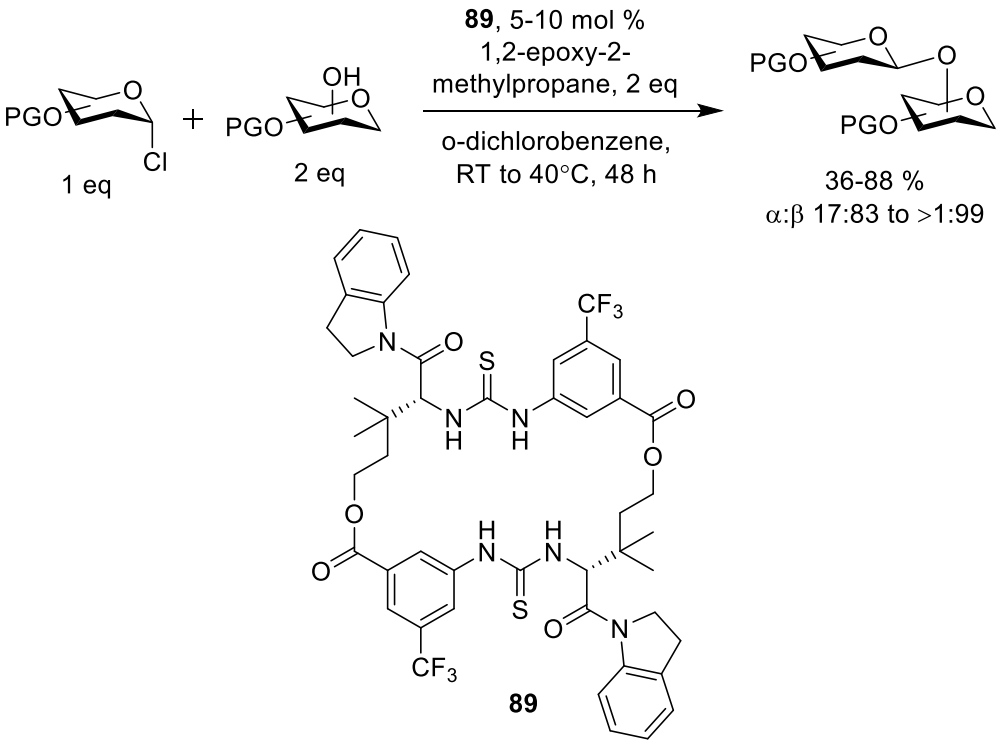
Scheme 22. *Glycosylation of galactals catalysed by thiourea* ***86*** *furnishes 2-deoxyglycosides with complete α selectivity.*[108]

The authors undertook a mechanistic investigation of the reaction mechanism using deuterated galactal **87**. When subjected to the reaction conditions using glycosyl acceptor **63**, 2-deoxyglycoside **88** was synthesised in 95 % yield, with a *cis* relationship between the newly formed C-2-H and C-1-O bonds, indicating a *syn* addition of the alcohol group to the α face of galactal **87** (**Scheme 23**). It was thus hypothesised that the reaction proceeds via addition of a proton from the alcohol-thiourea complex formed *in situ* to the less sterically hindered face of the galactal (**A**); to transiently produce tight ion pair (**B**), which will immediately go on to form deoxyglycoside **88** and liberate thiourea **86**. It is thought that there is a propensity for formation of the α anomer over the β anomer during the glycosidic bond forming step as a result of the anomeric effect, a lower energy steric interaction and a lower energy chair-like transition state. Note that here, as in the tetrahydropyranylation reported by Schreiner and co-workers,[107, 110] the thiourea was proposed to act as a dual hydrogen bond donor that binds to the oxygen atom on the alcohol.



Scheme 23. *Mechanistic pathway proposed by Galan and co-workers to explain the organocatalytic ability of thiourea* ***86*** *in the synthesis of 2-deoxyglycosides.*[108]

In 2017 Jacobsen and co-workers described an organocatalytic glycosylation attained through the use of a macrocyclic bis-thiourea as an organocatalyst, further demonstrating the versatility of thiourea derived organocatalysts.[111] The reaction uses a wide variety of α glycosyl chlorides as donors in combination with saccharide derived acceptors and novel macrocyclic (R,R) bis-thiourea **89** to prepare disaccharides with inversion of stereochemistry at the anomeric carbon, furnishing β glycosides with high selectivity (**Scheme 24**). Reactions usually took 48 h at RT or 40 ˚C and tended to produce the desired disaccharides in >60 % yield and with an α:β ratio of >1:9. The donor scope of the reaction in particular was excellent, as 12 different donors including fully oxygenated and deoxy or dideoxy glycosides were shown to work well under the reaction conditions. This generality was exploited to synthesise challenging 2-deoxy β glycosides and 1,2-*cis* β mannosides.



Scheme 24. *The macrocyclic bis-thiourea catalysed glycosylation strategy developed by Jacobsen and co-workers.*[111]

Surprisingly, the stereocontrol of the glycosylation was demonstrated to depend almost completely on the glycosyl donor configuration. Experiments were performed to independently vary the chirality of both the alcohol acceptor and the bis-thiourea organocatalyst. The results showed only very small changes in β selectivity, indicative of an S_N2 type mechanism. Experimental and computational studies into the mechanism established that bis-thiourea **89** is precisely suited to catalyse the reaction. Small modifications in, for instance, amide substituent or linker length caused a large decrease in yield and selectivity. The authors proposed a transition state structure as shown in **Figure 12**. The four NH protons of the bis-thiourea simultaneously form hydrogen bonds to the chlorine atom on the donor, whilst the amide carbonyl bond is able to form a hydrogen bond with the incoming alcohol. This allows a complex to form between the glycosyl chloride, alcohol and bis-thiourea that encourages an S_N2 type substitution.

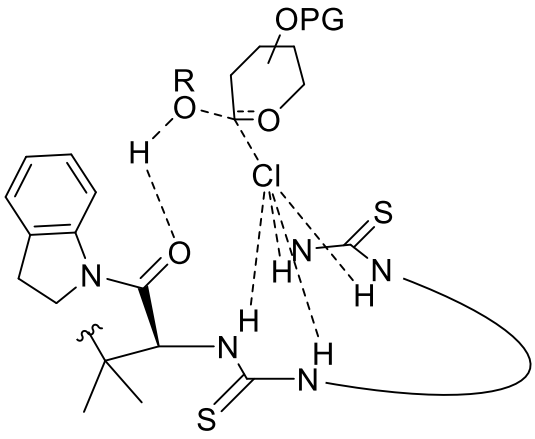
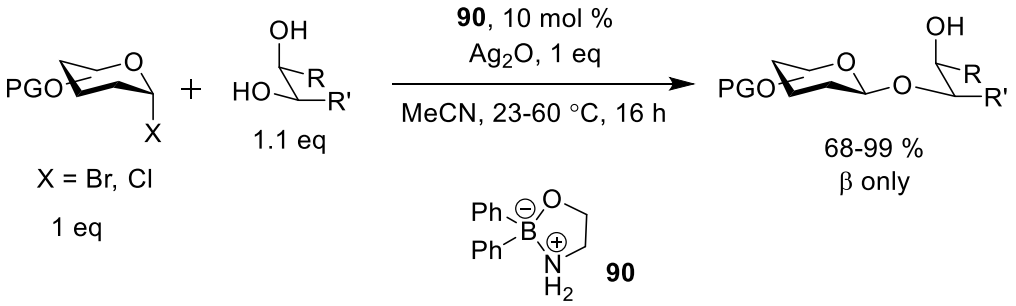


Figure 12. *Transition state complex proposed by Jacobsen and co-workers in the glycosylation of glycosyl chlorides mediated by bis-thiourea* ***89****.*[111]

Organoboron compounds possess the ability to reversibly make boron-carbon and boron-oxygen bonds, a useful feature for controlling stereochemistry, but also regiochemistry in glycosylation.[112, 113] In 2011, Taylor and co-workers reported a Koenigs-Knorr glycosylation of carbohydrates catalysed by a diphenylborinic acid derivative in combination with silver^{I} oxide.[114] Intriguingly, the reaction used saccharide derived glycosyl acceptors that only required protection at C-1 and C-6. As long as a single 1,2-*cis* diol moiety was present in the saccharide acceptor, total regioselectivity for the equatorial alcohol group in the *cis* diol was observed. Accordingly, α-halo glycosyl donors furnished disaccharides in excellent yields and complete β selectivity (**Scheme 25**). The substrate scope of the reaction was very good, with a range of seven glycosyl donors bearing ester and ether type protecting groups proving amenable to glycosylation with a number of mannose, galactose, fucose, arabinose and rhamnose derived glycosyl acceptors. The reaction utilised diphenylborinic acid **90** as an organocatalyst in combination with stoichiometric silver^{I} oxide to produce desired disaccharides in 16 hours. The excellent β selectivity achieved is perhaps not surprising in cases where the glycosyl donor had a participating protecting group at C-2 that may bias the formation of a 1,2-*trans* glycoside, however, the β selectivity is maintained even when non-participating benzyl protecting groups were employed. Furthermore, rigorous exclusion of moisture during the reaction was not necessary to maintain high yields. Mechanistic experiments undertaken led the authors to believe that catalyst **90** is in fact a precatalyst that is activated through displacement of ethanolamine by 1,2-*cis* hydroxyl groups present on the

saccharide glycosyl acceptor. An S_N2 type glycosylation was proposed in which a silver-halide interaction encourages attack by intermediate **91** (**Figure 13**). This glycosylation protocol was subsequently applied to the regioselective preparation of the cardiac glycoside natural product digitoxin.[115]



Scheme 25. *Regioselective glycosylation using diphenylborinic acid derivative* ***90*** *developed by Taylor and co-workers.*[114]

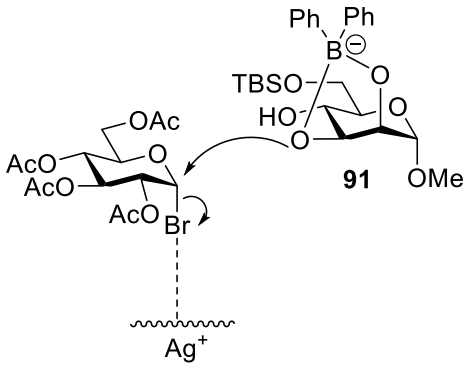
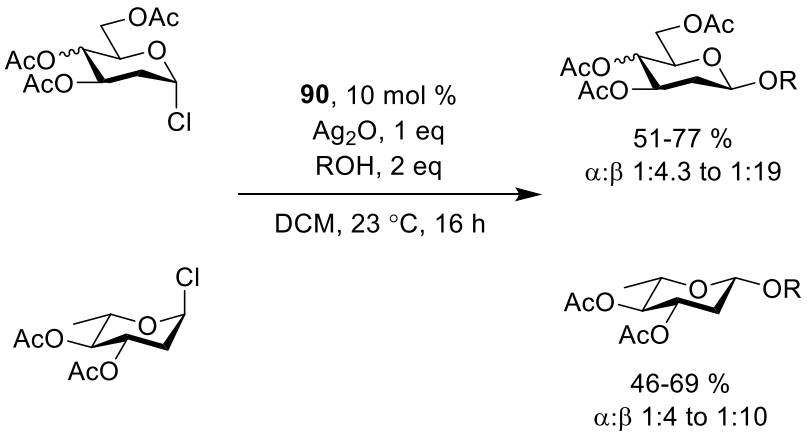


Figure 13. *Mechanistic rationale for observed selectivity in glycosylation catalysed by organocatalyst* ***90****.*[114]

In a subsequent publication, the authors built upon their previously reported work with organoboron catalyst **90** to prepare 2-deoxy- and 2,6-dideoxyglycosides.[116] Through the use of electron withdrawing acyl protecting groups installed on a 2-deoxy glycosyl donor with an anomeric α-chloro substituent, excellent regioselectivity was maintained whilst β selectivity remained highly favoured. Thus, the reaction conditions were successfully applied to three different galactosyl, glucosyl, and rhamnosyl derived donors using either 1,2- or 1,3-*cis* diol-bearing saccharide acceptors (**Scheme 26**).



Scheme 26. *Extension of the work by the Taylor group to encompass 2-deoxy and 2,6-dideoxyglycosyl donors in organoboron catalysed glycosylation.*[116]

2.6. **Glycosylation under Flow Conditions**

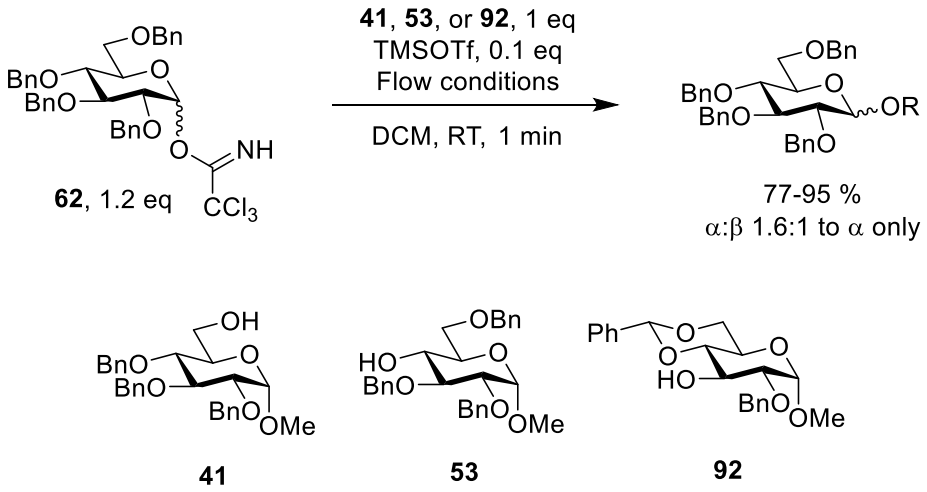
A further area of interest for the work described in this thesis is that of flow chemistry. Pumping solutions of reagents along tubing or etched channels and allowing chemical reactions to take place in a continuously flowing stream can offer significant advantages over conventional batch reactions. Reaction times can be controlled with exquisite precision, excellent mixing and heat transfer are possible and extremes in temperature and pressure are safely attainable. Flow chemistry has seen recent advancement in many areas of chemistry, including glycosylation.[117-122]

Performing chemical reactions in a continuously flowing stream, as opposed to a static reaction vessel as is traditional, offers several important advantages for reaction control, efficiency and the exploration of new regions of chemical reactivity.[117, 123] Rather than using the parameters of stoichiometry and reaction time to quantify a chemical reaction, one can instead use solution concentration and residence time. The residence time is the time that reactants spend in the reactor zone, given by the ratio of reactor volume to flow rate. Whilst not all chemical reactions are well suited to flow, especially those long reactions lasting several hours or days, reactions in flow possess several attributes that often make them superior to traditional batch reactions.

Firstly, due to the small dimensions of flow reactor tubing/channels in which the reaction occurs, the rate of mixing in flow reactors is generally far greater than that observed in batch reactors. This is because mixing is controlled by diffusion in a flow reactor as opposed to the turbulence-driven mixing usually required for batch reactions. A high mixing rate allows homogeneity of the reaction mixture that aids reaction rate. Furthermore, the rate of heat transfer is very high for the flow reactor compared to a batch reaction vessel such as a round-bottomed flask due to a high surface area to volume ratio, allowing more precise temperature control of the reaction. It is generally also possible, with the right setup, to heat the reaction solvent well above its boiling point safely. This allows reactions to take place within the superheated regime and often affords faster and cleaner reactions. Flow chemistry also lends itself to industrial scale chemical production, as scale-up and automation are reasonably straightforward in comparison with batch processes.[123] Perhaps one of the most useful aspects of flow chemistry in the research lab setting is the ability to telescope flow reactions together, permitting a multi-step synthesis in a fraction of the time it would take to do the same multi-step synthesis in batch. Moreover, telescoping reactions together means that a species can be synthesised within the confines of the flow reactor and immediately undergo a second reaction without ever having to be isolated. In this case, there is only ever a small quantity of the species in question present at any one time, allowing exploration of chemical space that would simply not be feasible in batch due to the safety concerns or decomposition of highly reactive, dangerous or short-lived chemicals. Flow chemistry has seen use in many synthetic chemistry disciplines, notably in the syntheses of medicinal compounds and natural products, but also for chemical glycosylation strategies. This section will describe several important developments in the literature for flow glycosylations.

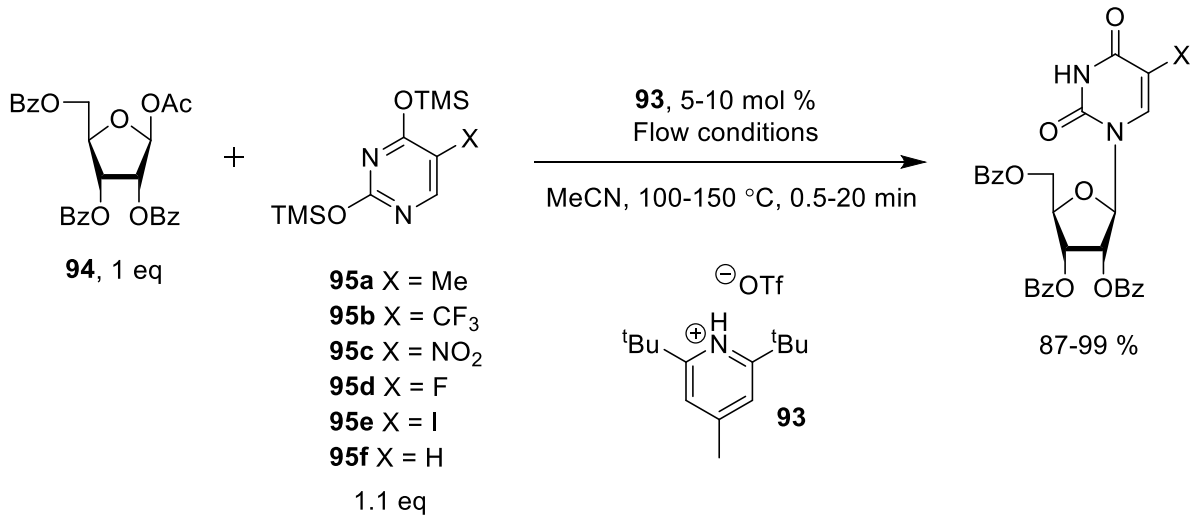
In 2014, Lay and co-workers described the experiments they carried out to optimise glycosylation of trichloroacetimidate and thioglycoside donors under flow conditions.[121] The aim was to evaluate the potential advantages and disadvantages of flow microreactor glycosylations as opposed to batch reactions. Batch reactions were performed under standard conditions, then comparative reactions in flow were undertaken, with appropriate optimisation of variables such as residence time and temperature. **Scheme 27** shows the results of the glycosylation of trichloroacetimidate donor **62** with several glycosyl acceptors **41**, **53** and **92**, under flow conditions using TMSOTf as promoter. Disaccharide products were

obtained in 77-95 % yields within 1 minute residence time at room temperature, giving slightly higher yields than their batch reaction counterparts. Stereoselectivities were fair to good, but generally showed little change on moving from batch to flow. Similar results were obtained for several other glycosyl donors bearing trichloroacetimidate or thionyl leaving groups. The flow paradigm proved amenable to scale-up, as evidenced by production of 0.44 g (90 % yield) of a disaccharide over 100 minutes. Furthermore, dry solvents and exclusion of moisture were not strictly required for the flow reaction, reagent grade solvents were sufficient to allow high yields to be attained. The study culminated in a multi-step flow synthesis of a trisaccharide from constituent monosaccharides in 51 % overall yield.



Scheme 27. *Glycosylation under flow conditions using glycosyl donor* ***62*** *as reported by Lay and co-workers.*[121]

A fascinating area in which glycosylation plays a pivotal part is in nucleoside synthesis. In 2011, Jamison *et al*. disclosed an organocatalytic Brønsted acid catalysed glycosylation of a ribofuranose donor with a number of different nucleobases to prepare ribonucleosides using continuous flow techniques.[124, 125] Ribonucleosides are often synthesised using the Vorbrüggen variation of the silyl-Hilbert-Johnson reaction. This method uses a Lewis acid in stoichiometric quantities as a promoter, leading to difficulties with functional group tolerance and the generation of significant amounts of chemical waste. By employing 2,6-di-*tert*-butyl-4-methylpyridinium triflate **93** as an organocatalyst, suitably protected ribofuranose **94** was *N*-glycosylated using various uracil derived nucleobases **95a-f** (**Scheme 28**), in addition to several guanine, cytosine and adenine derived nucleobases.

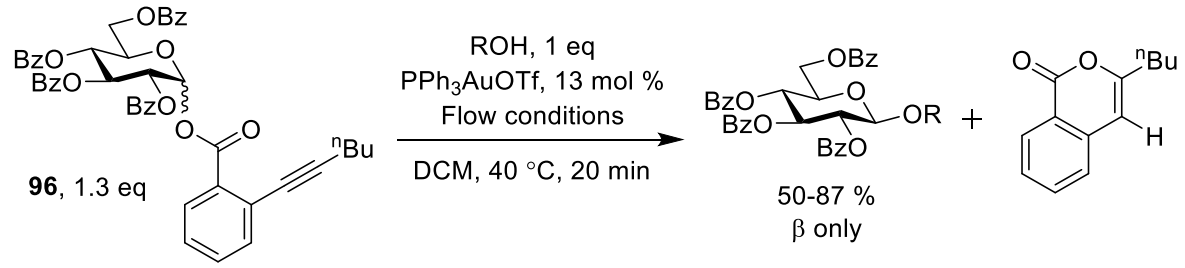


Scheme 28. *Pyridinium derived Brønsted acid* ***93*** *catalyses the synthesis of ribonucleosides under flow conditions.*[124]

The reaction furnished the desired products in excellent yields and with total β selectivity. This outcome may be ascribed to the anchimeric assistance of the neighbouring benzoyl group at C-2 of the glycosyl donor. The authors noted that the glycosylation worked well in flow if the temperature was regulated to maintain the solubility of reactants and products, but also worked in batch with microwave irradiation to stimulate the reaction. By scaling up the reaction to a commercial flow system, multi-gram quantities of ribonucleosides were produced in high purity with minimal optimisation. Screening experiments of the organocatalyst determined that the characteristics of both the cation and the anion were essential to ensure high yields of product. Further work by the authors exploited this glycosylation in a telescoped multi-step flow synthesis to prepare a number of 5'-deoxyribonucleoside pharmaceuticals.[126]

In the final report that shall be considered in this section, Seeberger and co-workers brought the synthetic potential of gold catalysis to continuous flow methodology.[127] The alkynophilicity of gold has been previously described in this report in the context of propargyl and *S*-but-3-ynyl donors for glycosylation. Subsequent to those reports, the advent of glycosyl *ortho*-alkynylbenzoates as a new class of glycosyl donors amenable to gold activation appeared in the literature. The Yu group developed this gold(I) catalysed glycosylation into a powerful strategy that allows the synthesis of glycosides in excellent yields.[128-130] In 2015, the

Seeberger group successfully applied the glycosylation of glycosyl *ortho*-alkynylbenzoates through gold(I) catalysis to the flow regime. Several differentially protected donors were screened using the catalyst PPh_3AuOTf at 13 mol % stoichiometry to furnish products in good to excellent yields over reasonably short residence times of 20-30 minutes. Using anchimerically participating donor **96**, five product glycosides were prepared in 50-87 % yield and total β selectivity (**Scheme 29**).



Scheme 29. *Glycosylation of glycosyl ortho-alkynylbenzoates through gold(I) catalysis in flow.*[127]

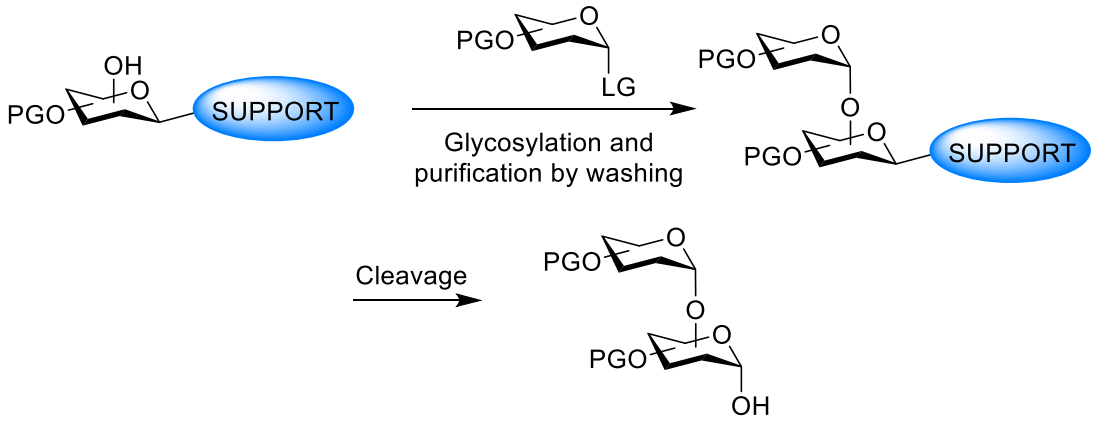
After establishing optimal reaction conditions using donor **96**, a donor bearing non-participating benzyl protecting groups in place of benzoyl groups was tested to ascertain the α:β selectivity of the reaction. Whilst yields remained very high in most cases, selectivities were generally poor to moderate. Furthermore, 2-amino and 2-deoxy glycosyl donors were successfully glycosylated, demonstrating the versatility of the method. The mechanism of the reaction has been investigated extensively by Yu and co-workers[128] and features a gold-catalysed activation of the anomeric leaving group, encouraging S_N2 substitution at the anomeric centre. However, the mechanism is somewhat intricate and the S_N1-S_N2 character depends upon the specific reaction conditions.

The discipline of glycosylation using flow conditions is still in its infancy, however, the scope for rapid and clean reactions is unquestionable. Additionally, a major obstacle in glycosylation optimisation is the synthesis of precursor donor and acceptor glycosides, each of which frequently requires a substantial number of synthetic steps to prepare. Flow microreactors minimise this issue as only very small quantities of reagent are required in order to perform many reactions. The application of highly efficient catalytic processes to the flow regime is sure to be an area that sees literature attention over the coming years.

2.7. Glycosylation Using Imidazolium Based IL Supports

Ionic liquids (ILs) are a versatile and synthetically useful class of compounds that have found use in diverse fields, for instance as green solvents, catalysts or co-catalysts and ligands.[131-136] ILs make excellent candidates for greener alternative solvents to volatile organic media, owing to their negligible vapour pressure and lack of flammability. They therefore pose a much lower risk to human health and the environment, particularly the atmosphere, where volatile organic solvents can cause significant damage through photochemistry. However, ionic liquids may also be used as soluble supports through covalent linkage to a desired substrate, facilitating easy analysis and purification of the substrate as chemical transformations are performed upon it.

In supported oligosaccharide synthesis, the oligosaccharide chain is firstly attached to a support, that can be either solid or soluble. Reactions are then performed upon the attached carbohydrate, for example glycosylations to lengthen the oligosaccharide chain or protecting group manipulations. After each reaction, purification can be achieved very easily by simply washing away all non-supported material including side products or excess reagents. When the desired oligosaccharide has been constructed, the product can be cleaved from the support chemoselectively to liberate the free desired compound (**Scheme 30**).[137]



Scheme 30. *General representation of a supported oligosaccharide synthesis strategy.*

Ionic tags (I-Tags) are ionic functional groups that can be used as a solution phase purification handle and a MS reaction progress reporter.[136] These types of labels often bear

quaternary amine groups and are covalently linked to one of the monosaccharide units of a growing oligosaccharide target. I-Tags are well suited to serve as supports for oligosaccharide synthesis for several reasons. Firstly, mass spectrometry relies on molecular ions that bear a positive charge for detection. Since I-Tagged sugars already bear a full positive charge, they do not rely on the ionisation technique to cause ionisation like neutral molecules do, they need only be vaporised. Therefore, since 100 % of the sample can theoretically be detected by the mass spectrometer, minute quantities of material can be easily analysed by MS.[138] Thus, where for instance an NMR spectrum of a reaction mixture may be difficult to interpret due to many overlapping signals, the mass spectrum will generally show all neutral compounds as part of the baseline whilst I-Tagged compound detection limits are much lower. Secondly, in contrast to solid supports, I-Tag supports are soluble in more polar organic solvents such as DCM and acetonitrile and thus supported reactions can take place under homogeneous solution phase conditions. This means that smaller quantities of reagents can be used compared to solid supported strategies, whilst conventional analysis techniques such as NMR spectroscopy, MS and HPLC can be used to track reaction progress.

Thirdly, the presence of a permanent positive charge dramatically increases the net polarity of the molecule. This property changes the propensity for dissolution of the I-Tagged sugar in different solvents according to the polarity of the solvent. Hence, owing to the polarity difference between protected neutral sugars and sugars bearing I-Tags, purification of the I-Tagged sugar may be achieved through trituration with various combinations of solvents or a biphasic workup. This becomes especially useful when purifying a protected I-Tagged sugar after a glycosylation reaction. Apolar solvents such as hexane and diethyl ether (or a combination thereof) may be used to dissolve and wash away protected neutral sugar molecules, for example unreacted or hydrolysed glycosyl donor, whilst the product I-Tagged sugar is too polar to be dissolved in these solvents. Conversely, water, being an extremely polar solvent, may be used to wash away very polar impurities such as mineral acids or inorganic salts, whilst the I-Tagged sugar, if appropriately protected, is too apolar to be dissolved in water. This approach allows purification of glycosylation products with no requirement for time-consuming column chromatography as would be traditionally required (**Figure 14**).

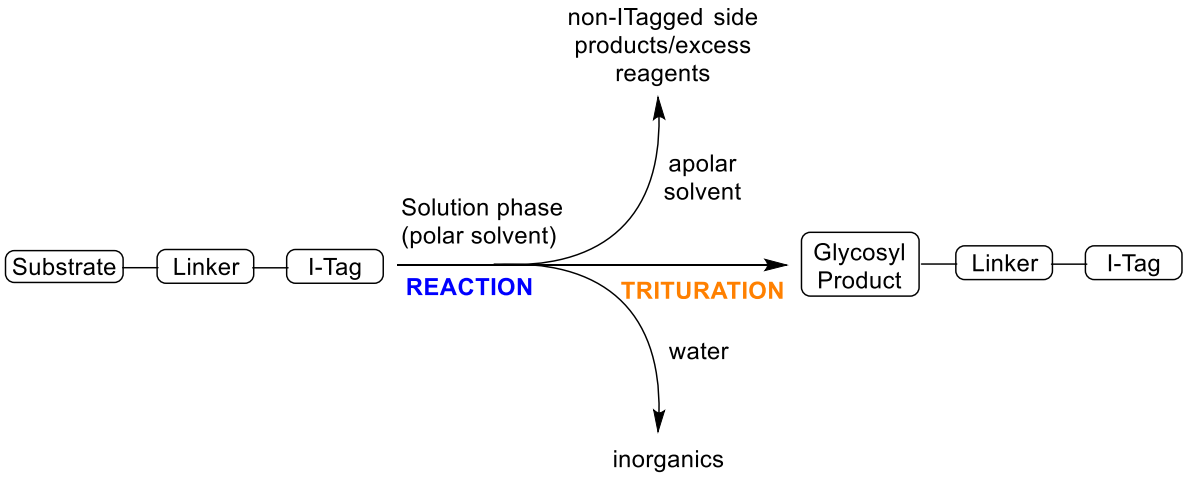
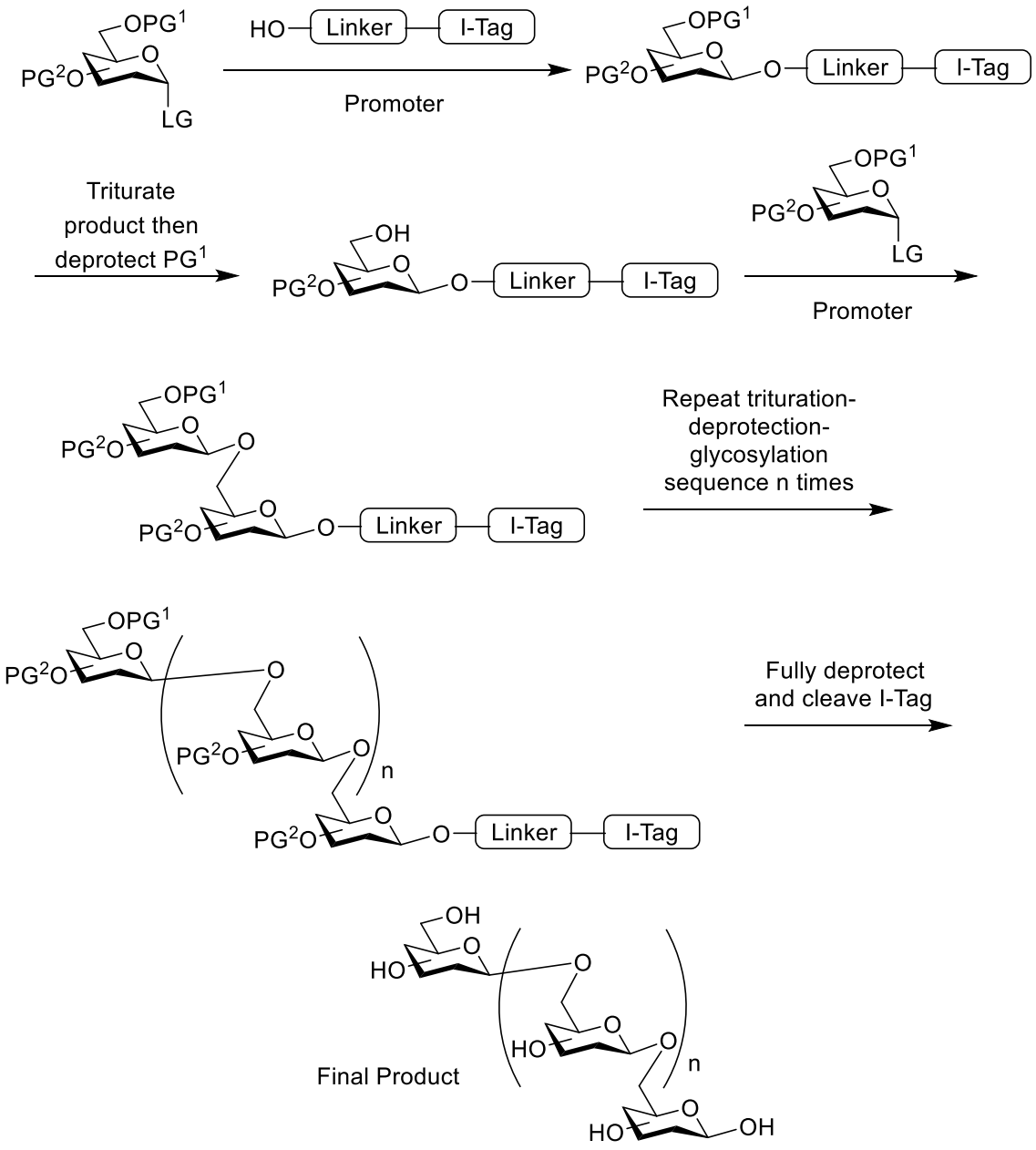


Figure 14. *Strategy for trituration-based purification of oligosaccharides using an I-Tag support.*

Finally, an appropriately designed glycosyl donor bearing orthogonal or semi-orthogonal protecting groups can be used to rapidly construct an oligosaccharide through a repeated glycosylation-deprotection-trituration sequence. Consider the example shown in **Scheme 31**. Attaching an I-Tag to an appropriate glycosyl donor gives a product amenable to purification by simple trituration. Deprotection of protecting group PG^1 furnishes a free alcohol that can act as a glycosyl acceptor for a glycosyl donor in a glycosylation reaction. Continued iterations of the glycosylation-deprotection-trituration sequence permits the synthesis of complex oligosaccharides quickly and efficiently. Final universal deprotection yields a free oligosaccharide if required. This may include removal of the I-Tag if a cleavable linker is used.

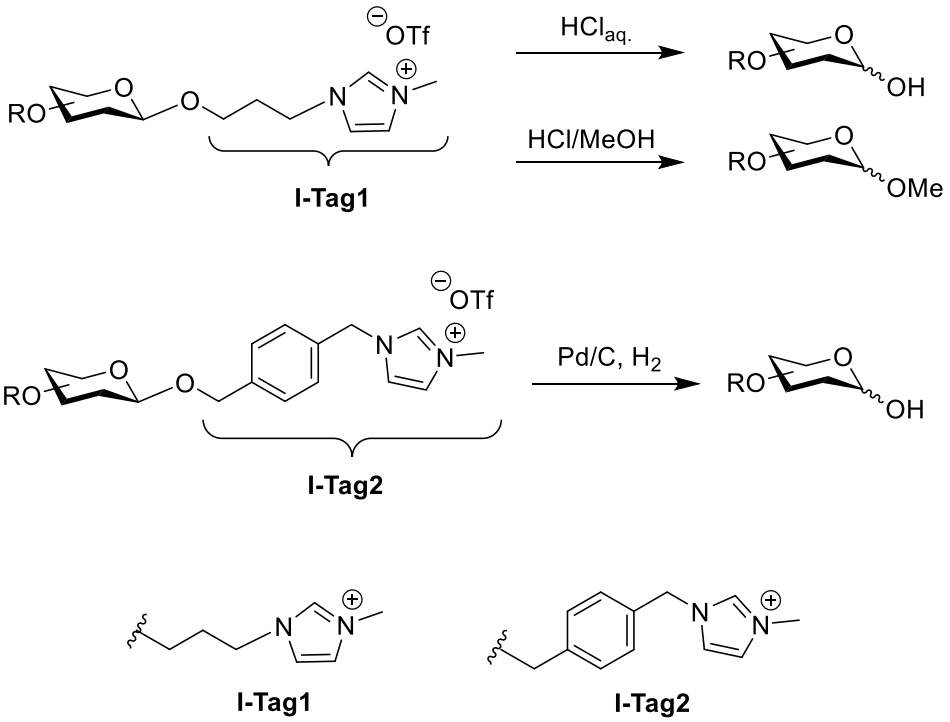


Scheme 31. *Representation of the strategy used to construct complex oligosaccharides using an I-Tag motif as a tool in the facile purification of synthetic intermediates.*

Earlier reports of I-Tag supported oligosaccharide syntheses tended to attach the I-Tag via an ester linkage to the glycosyl donor.[139-143] However, this strategy, though synthetically useful, had several drawbacks. Ester protecting groups are used commonly in carbohydrate chemistry and thus attaching the I-Tag via an ester linkage prevents the use of common protecting groups such as acetates and benzoates, because the removal of such protecting groups, usually achieved through base catalysed esterification or hydrolysis, is also

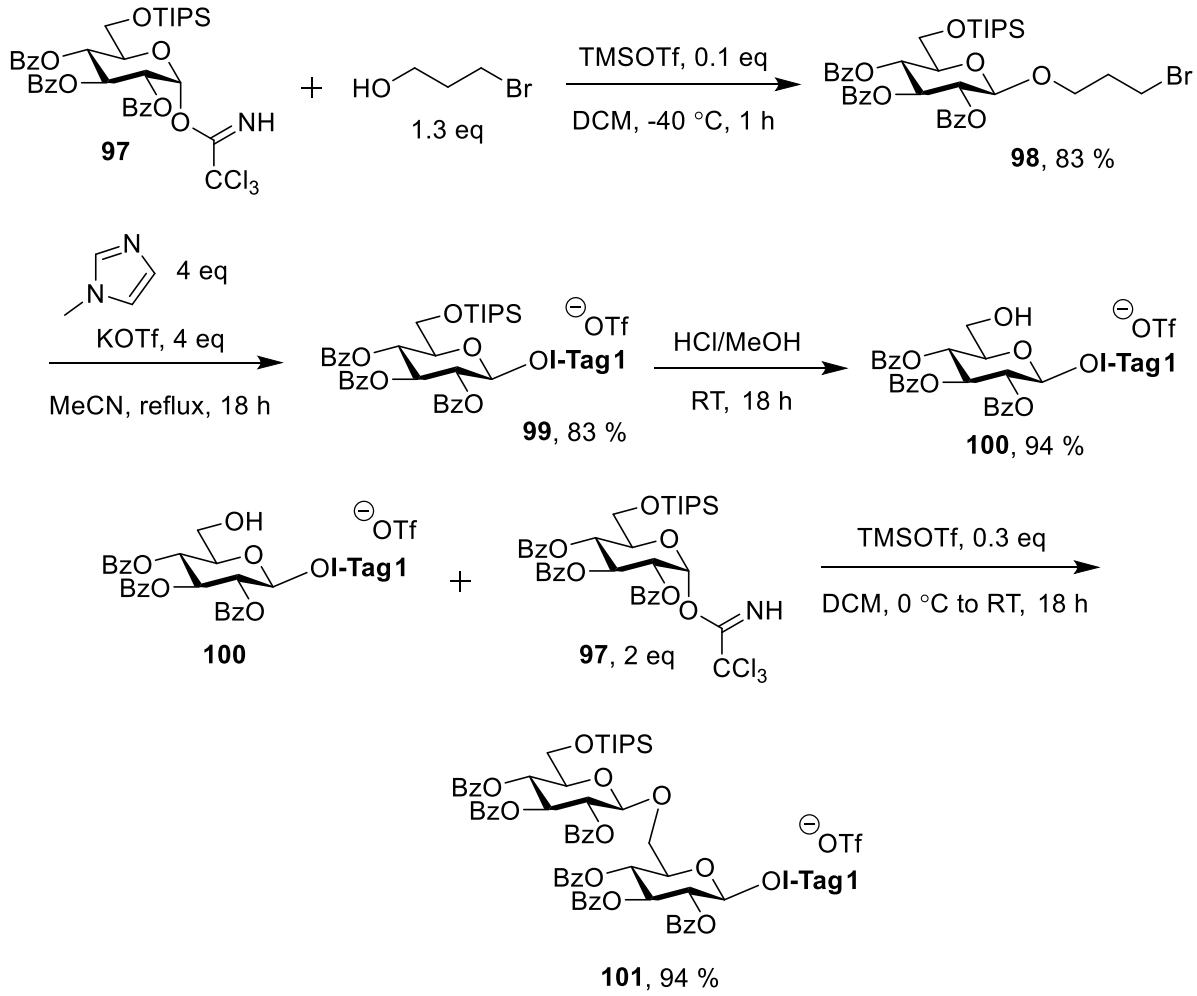
likely to cleave the I-Tag at an inconvenient time. Secondly, installing the I-Tag on the donor is likely to increase the number of steps required in the overall synthesis, since the I-Tag must be removed after each glycosylation performed which may hamper the efficiency of oligosaccharide synthesis using this approach. Lastly, it is common to use an excess of donor in glycosylations to encourage a higher yield of product. Excess donor often becomes hydrolysed or undergoes side reactions due to the inherent anomeric reactivity of the donor. The products of such undesired reactions are usually removed during the purification stage, but if they bear I-Tag functionality, it is impossible to separate them from the I-Tagged desired product by trituration.

As a result of these concerns, it has become more common in the literature to install the I-Tag on the glycosyl acceptor, often at the anomeric carbon, via an ethereal linkage. Such an approach was taken by Galan and co-workers in 2011 when they disclosed the so-called ICROS (Ionic Catch and Release Oligosaccharide Synthesis).[144] Two different I-Tags were reported, shown in **Scheme 32**. **I-Tag1** featuring a propyl linker is a highly robust group, able to survive common reaction conditions used in carbohydrate manipulation. Cleavage may be achieved through acid catalysed Fischer glycosylation in water or methanol. However, such cleavage conditions may also break other glycosidic bonds in an oligosaccharide, resulting in truncated structures.[145] To avoid this problem, **I-Tag2** was developed using a benzylic linker that may be cleaved using palladium catalysed hydrogenolysis whilst leaving other glycosidic bonds in the saccharide intact.



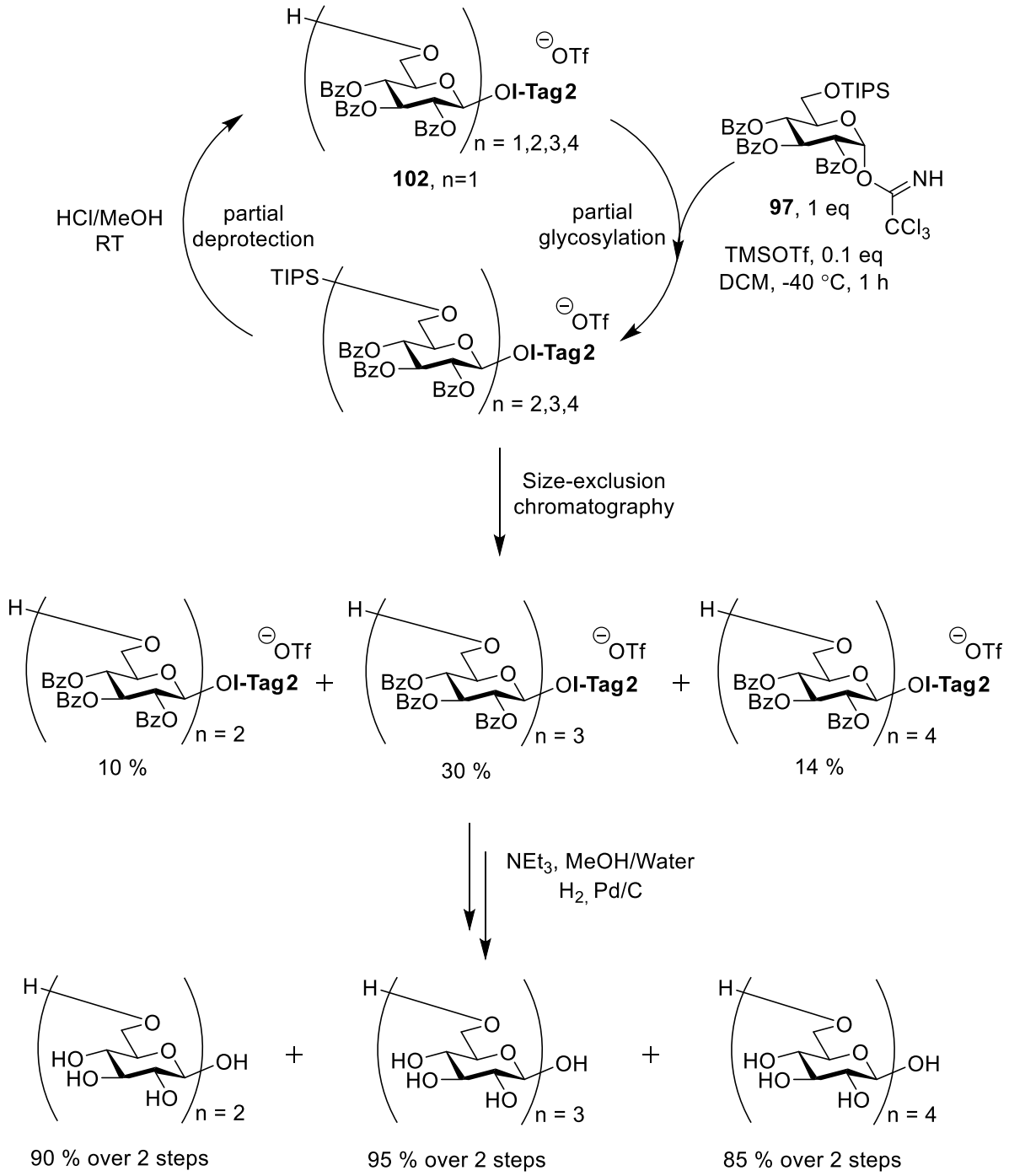
Scheme 32. *The two I-Tags developed by Galan and co-workers and strategies for orthogonal I-Tag cleavage.*[144]

Scheme 33 shows the strategy used for installation of **I-Tag1** and subsequent I-Tag oligosaccharide synthesis to construct β 1,6-D-glucans found naturally in fungal cell walls. The multi-step synthetic strategy begins with glycosylation of orthogonally protected glycosyl trichloroacetimidate donor **97** with glycosyl acceptor 3-bromopropan-1-ol to furnish product **98** in 83 % yield over 1 hour. Reacting **98** with 1-methylimidazole in the presence of potassium triflate gave I-Tagged sugar **99** in 83 % yield after 18 hours. Deprotection of the *O*-6 triisopropylsilyl ether protecting group was completed using a solution of hydrochloric acid in methanol over 18 hours to produce I-Tagged glycosyl acceptor **100** bearing a free alcohol group in 94 % yield. Finally, glycosylation of **100** with donor **97** using TMSOTf as the promoter was completed over 18 hours to give I-Tagged disaccharide **101** in 94 % yield. The silyl ether deprotection and glycosylation sequence was repeated twice more to afford a tetrasaccharide in 69 % yield. Following each deprotection and glycosylation reaction after **I-Tag1** was installed, purification was achieved through trituration using Et_2O/*n*-hexane.



Scheme 33. *ICROS strategy developed by Galan and co-workers to synthesise β 1,6-D-glucans.*[144]

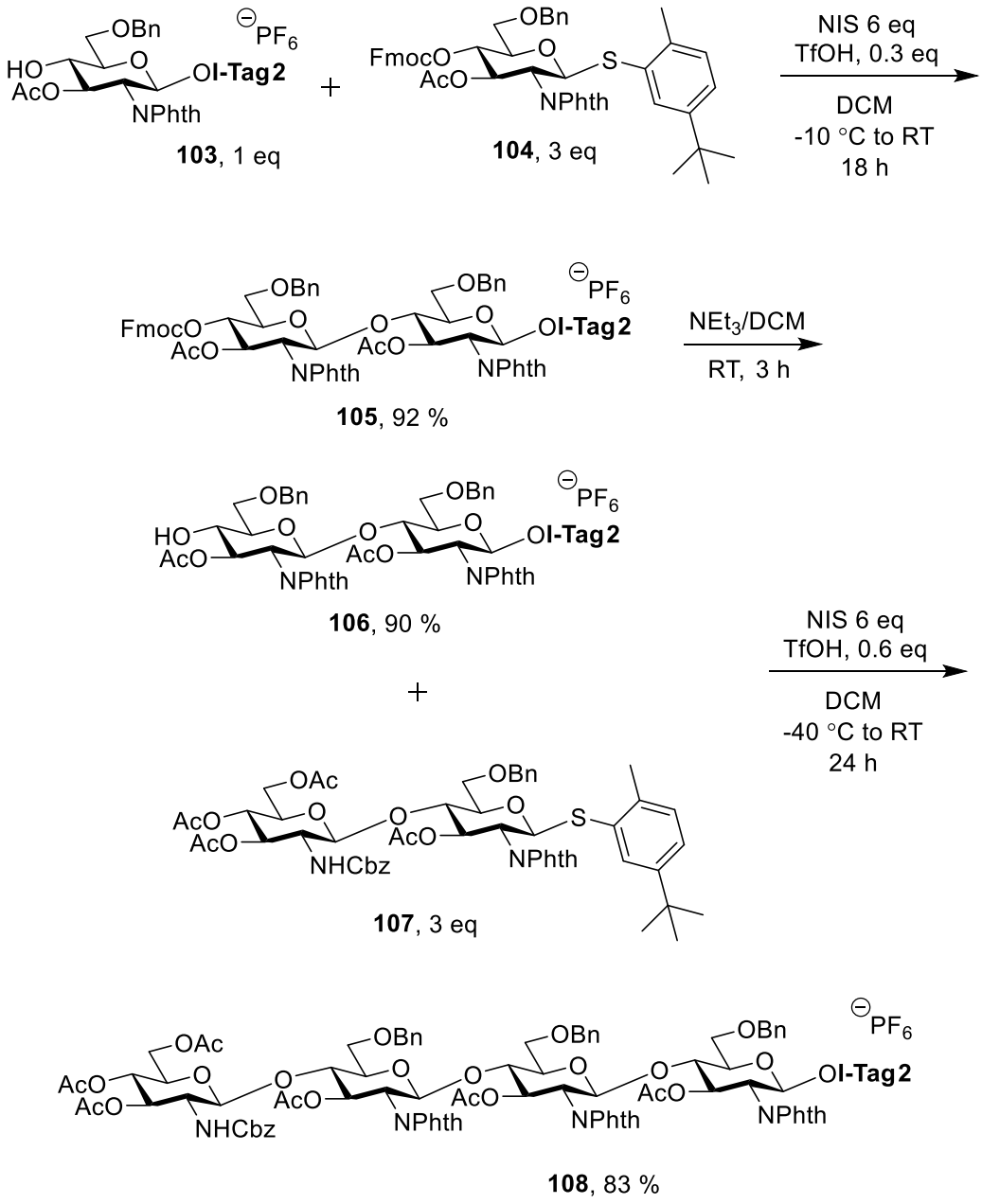
This work was later extended to encompass combinatorial oligosaccharide synthesis, in which several oligosaccharide targets were synthesised over a few days.[146] Over three cycles of partial glycosylation and partial deprotection of I-Tagged glycosyl acceptor **102** a di-, tri- and tetrasaccharide were synthesised as shown in **Scheme 34**. Purification of intermediates was performed by washing with Et_2O:hexane 1:1, whilst reaction progress was monitored using HPLC, MALDI MS and ^1H NMR spectroscopy to control the degree of glycosylation and silyl ether deprotection at each step of the cycle. Following completion of the third cycle, size-exclusion chromatography was used to separate the individual protected oligosaccharides in good yield when considered cumulatively as 54 % total yield over 6 steps. Base catalysed removal of benzoyl groups followed by catalytic hydrogenolysis afforded the globally deprotected glycosides in excellent yields.



Scheme 34. *Combinatorial ICROS protocol reported by Galan and co-workers. A mixture of di-, tri- and tetrasaccharides were synthesised, then separated using size-exclusion chromatography. Global deprotection yielded globally deprotected pure glycosides.*[146]

Beau and co-workers also recently reported the use of I-Tag supports for chitooligosaccharide synthesis.[147] The same cationic I-Tag structure **I-Tag2** was used for the ionic liquid support, but with a hexafluorophosphate counterion rather than triflate. Their synthetic approach towards orthogonally protected β-1,4-tetrasaccharide **108** is outlined in **Scheme 35**. Tetrasaccharide **108** is a key intermediate in the preparation of naturally occurring lipo-chitooligosaccharides and *N,N,N*-trimethylglucosamine-chitotriomycin, compounds that exhibit noteworthy biological activities. I-Tagged glycosyl acceptor **103** was smoothly glycosylated with thioglycoside donor **104** to afford disaccharide **105** in excellent yield and complete β stereoselectivity. Deprotection of the Fmoc group with triethylamine afforded **106** in 90 % yield, which was then used as a glycosyl acceptor in the reaction with donor **107** in a 2 + 2 glycosylation to furnish **108** in 83 % yield. In each case the I-Tagged product was purified by solvent washes. However, the authors noted that final deprotection of the tetrasaccharide was challenging, being impossible to purify through solvent washes. They attributed this unusual behaviour to the particular physicochemical properties of the oligo-chitin acetamido structure bearing an I-Tag.

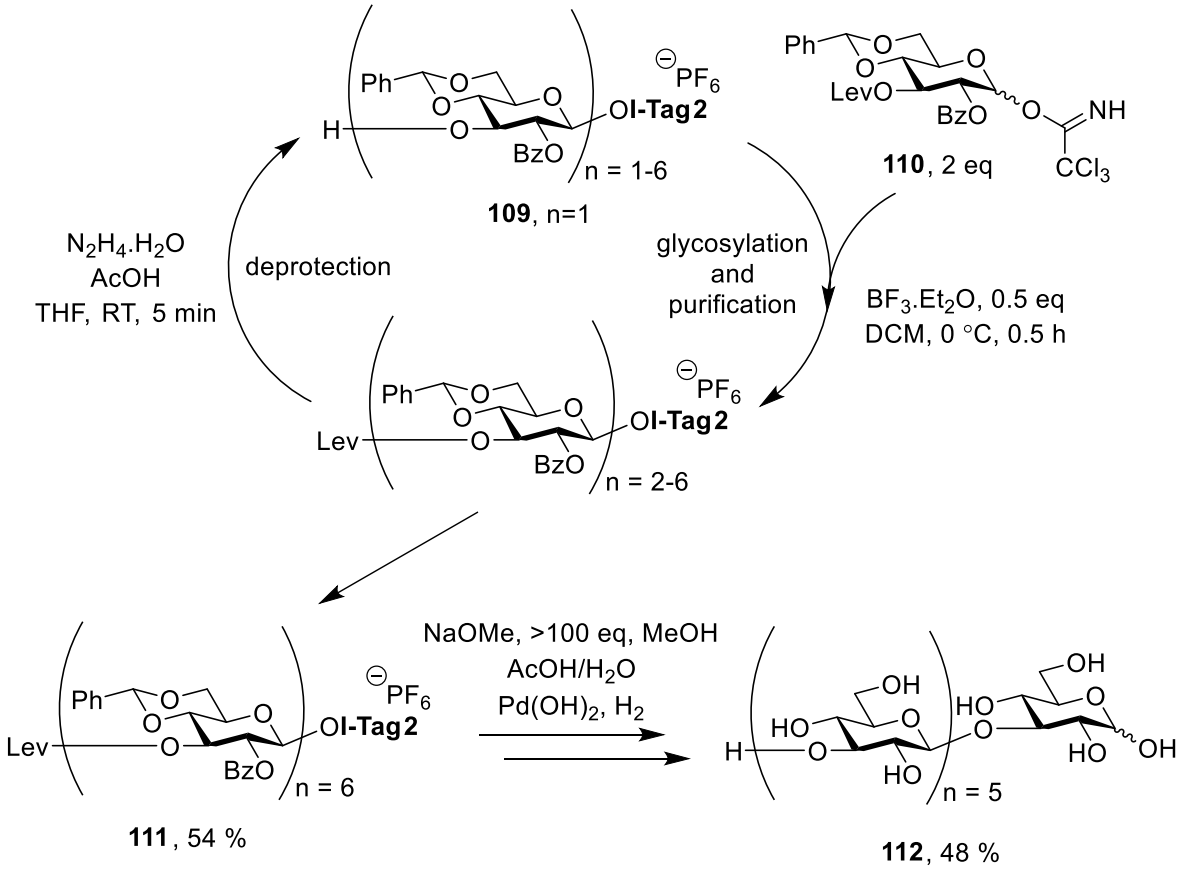
The use of **I-Tag2** was exploited further by Li and co-workers in the preparation of a synthetically challenging β-1,3-glucan hexasaccharide.[148] β-1,3-glucans, known as laminarin polysaccharides, are found throughout many natural sources, for instance in yeast cell walls. They have garnered synthetic interest as a result of their antibacterial, antitumour and immunostimulating medicinal properties. In their strategy, the researchers described how orthogonally protected donor **110**, bearing a levulinyl group that may be deprotected using hydrazine, could be used to efficiently construct the desired hexasaccharide in just 15 hours. Initial reactions by the authors using TMSOTf as the glycosylation promoter proved unsuccessful, due to unintended formation of the TMS ether at the glycosyl acceptor hydroxyl group during glycosylation. However, $BF_3.Et_2O$ was found to facilitate smooth glycosylation with no significant side products.



Scheme 35. *I-Tag supported synthesis of β-1,4-chitotetrasaccharide* ***108*** *as reported by Beau and co-workers.*[147]

Scheme 36 shows the iterative oligosaccharide preparation approach employed by the Li group. Monosaccharide glycosyl acceptor **109** bearing **I-Tag2** was glycosylated using donor **110**, with simple chromatography-free purification. Rapid levulinyl deprotection followed to regenerate a glycosyl acceptor bearing a free hydroxyl group. The cycle continued several times to form protected hexasaccharide **111** in 54 % overall yield, with an average

yield of greater than 90 % per step. Final global deprotection afforded unprotected hexasaccharide **112** in 48 % yield over 3 steps.



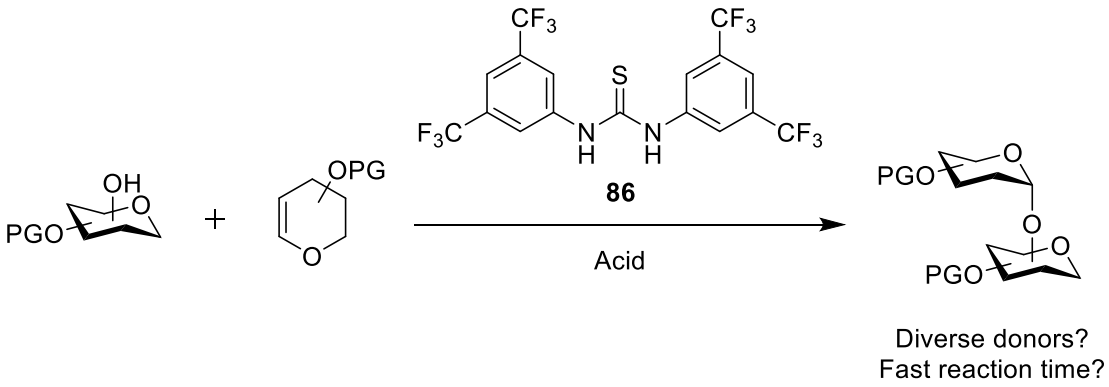
Scheme 36. *Repeated sequence of glycosylation, purification and deprotection afforded β-1,3-glucan laminarihexaose using I-Tag support.*[148]

Whilst I-Tag supported oligosaccharide synthesis only emerged fairly recently, it is rapidly becoming a useful tool in the carbohydrate chemist's repertoire for tackling challenging target molecules. The simple purification opportunity permitted through the I-Tag support saves a great deal of time, however, the synthesis of oligosaccharides is ultimately a highly laborious endeavour that would benefit from further innovations to expedite the process.

3. Project Aims

The introduction section describes state-of-the-art work reported in the scientific literature for the preparation of diverse and often biologically relevant carbohydrates. However, it is clear that despite considerable effort, this is still a very difficult and protracted endeavour. Two of the key obstacles that hamper expediency in oligosaccharide synthesis are firstly, stereocontrol of the glycosylation reaction and secondly, tedious and time-consuming reactions and chromatographic purifications. The goal of this PhD project was to build upon previous work in overcoming these hurdles. In the first part of the project, efficient, stereoselective, catalytic routes to challenging 2-deoxyglycosides that are simpler and more versatile than those that had been reported previously were explored. The second part of the project was to investigate whether, given conditions that allow for stereoselective glycosidic bond formation, the application of continuous flow techniques could permit faster and more expedient oligosaccharide synthesis.

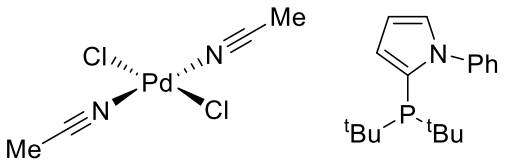
As described previously, the Galan research group developed an organocatalytic α-selective 2-deoxygalactoside synthesis that used thiourea **86** to effect glycosylation. Whilst a very powerful transformation, the reaction had some drawbacks, namely that only galactal type donors were suitable for the reaction and reactions generally took 24 – 48 hours in refluxing DCM to reach completion. To address these problems, it was hypothesised that the synergistic combination of thiourea **86** with a Brønsted acid as dual organocatalysts may permit a more efficient, fast and widely applicable glycosylation than either a thiourea or acid alone (**Scheme 37**).



Scheme 37. *Proposed glycosylation of glycals to form 2-deoxyglycosides through cooperative catalysis using a thiourea and a Brønsted acid.*

Simultaneously, the possibility of using transition metal catalysis for glycosylation of glycals was evaluated. More specifically, palladium catalysis applied to 2-deoxyglycoside preparation from glycal donors had not been attempted in the literature prior to our work. It was thought that choosing an appropriate ligand in conjunction with the palladium metal may temper the Lewis acidity of the metal, thus biasing reactivity towards 2-deoxyglycoside synthesis as opposed to Ferrier rearrangement. Both the organocatalytic and palladium catalysed glycosylation projects were contributed to during the course of the PhD studies.

Firstly, a library of glycosyl acceptors and donors were prepared for use in the glycosylation reactions. With respect to the organocatalytic glycosylation project, a potential Brønsted acid was screened for its ability to synergistically catalyse the glycosylation. Also, a reaction using deuterated methanol as the glycosyl acceptor was explored in order to provide mechanistic information about the glycosylation. However, involvement with the palladium catalysed glycosylation project formed the bulk of early work during my PhD studies. By the time my work on the project began, a number of metal complexes and ligands had already been screened for their ability to catalyse coupling of glycals and alcohols and a “hit” had been discovered. The palladium(II) species bis(acetonitrile)dichloropalladium(II) **113** was found to have some activity with the sterically bulky phosphine ligand 2-(di-*tert*-butylphosphanyl)-1-phenyl-1H-pyrrole **114**.



113 **114**

Figure 15. *The active palladium species **113** and ligand **114** identified as being cooperative in the glycosylation reaction.*

The objectives of my work throughout this stage of my PhD research were firstly to investigate the activity of the catalyst and optimise the reaction by altering experimental variables including solvent, concentration, reaction temperature and duration and stoichiometry of reactants. Secondly, the tolerance of the methodology for different glycosyl acceptor alcohols – both sugars and other simple alcohols was probed. Both the thiourea-Brønsted acid organocatalysis project and the palladium catalysis project were ultimately successful, culminating in publications published during the course of my PhD.[149, 150]

As time went on, continuous flow synthesis was surveyed as a strategy to increase efficiency and reproducibility of glycosylation reactions, whilst reducing the time required for complete reaction. Thus, attempts were made to take glycosylation protocols that had been developed within the Galan group as a batch reaction and translate the reaction into the continuous flow regime, hopefully improving the reaction by doing so.[123, 151] At this point in time, the gold(I) catalysed glycosylation of glycal donors using gold catalyst **70** previously outlined in the introduction section **2.4.** (**Scheme 20**) had very recently been developed. The glycosylations had been performed in batch, however, it appeared that if suitable optimisation was performed, the reaction conditions could be amenable to flow.[99] Therefore, investigations into glycosylation in the flow regime with this gold(I) catalysed protocol began.

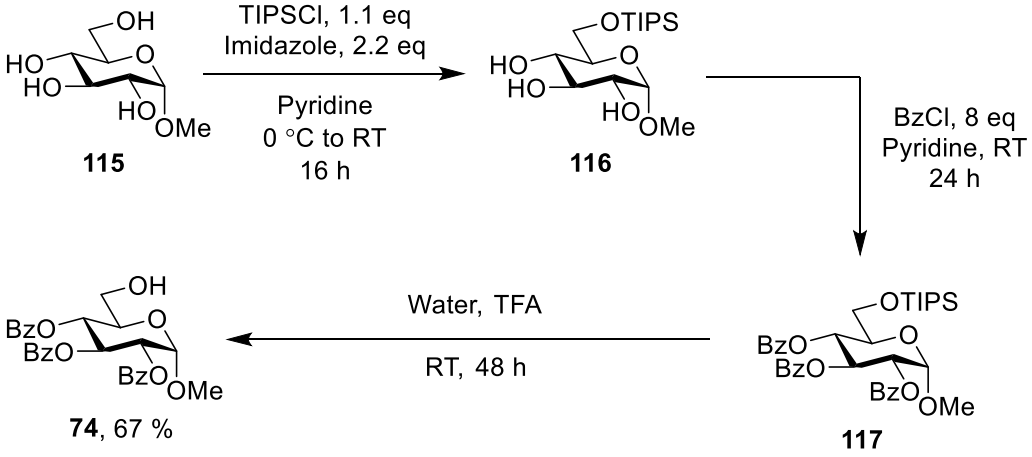
Over time, another flow glycosylation opportunity presented itself that showed considerably more promise. The use of carbohydrates bearing I-Tags as glycosyl acceptors for glycosylation was probed in the manner depicted previously in **Scheme 31**. Whilst I-Tags have been pioneered in the Galan laboratory with batch reactions as described in the introduction, they have never been applied to the flow regime.[144, 146] Thus, the aim was to translate known batch reactivity using I-Tags to flow glycosylation.

4. Results and Discussion

4.1. Synthesis of Starting Materials

4.1.1. Glycosyl Acceptor Syntheses

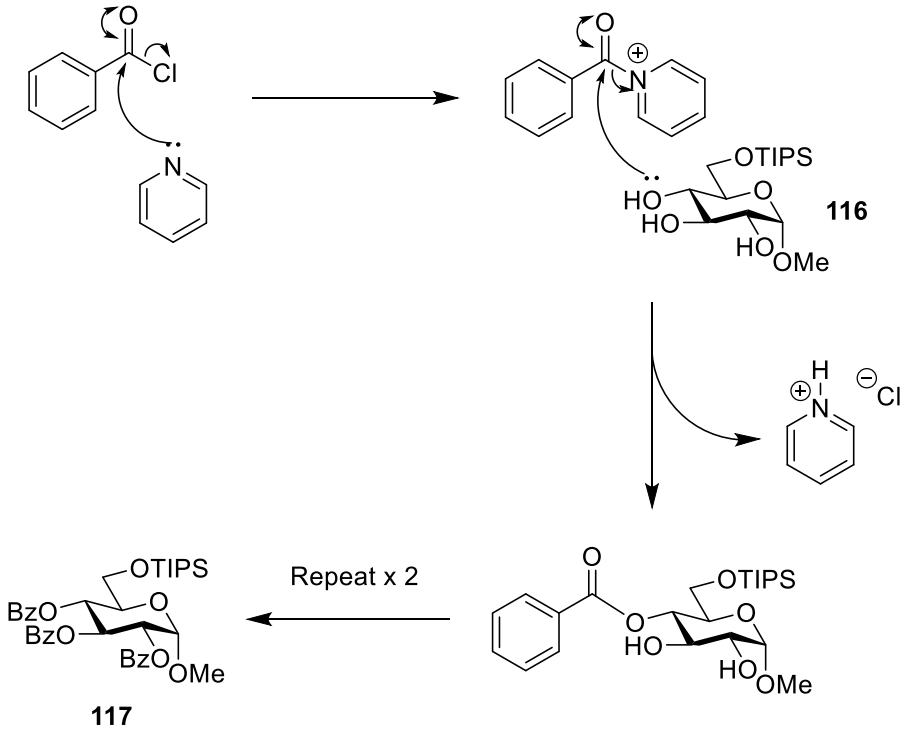
At the beginning of the project, a range of glycosyl acceptors were synthesised in multi-gram quantities for use in catalytic glycosylation experiments. However, the majority of the chemistry used in these syntheses is well established, therefore only a few of the key preparations will be discussed in detail. For full experimental details of the library of acceptors that were prepared, please refer to the experimental section.



Scheme 38. *Synthetic route from methyl α-D-glucopyranoside **115** to methyl 2,3,4-tri-O-benzoyl-α-D-glucopyranoside **74**.*

The preparation of C-6 primary glycosyl acceptor **74** begins with commercially available, cheap methyl α-D-glucopyranoside **115** (**Scheme 38**). Silylation of the primary alcohol using triisopropylsilyl chloride and imidazole in anhydrous pyridine proceeded smoothly over 16 h at room temperature. The large steric bulk of the three triisopropyl groups bonded to the silicon atom and the low reaction temperature help to ensure that only the most reactive primary C-6 alcohol undergoes silylation, leaving the more sterically hindered secondary C-2,3,4-OH groups unreacted. In the same reaction pot an excess of benzoyl chloride is added. The electrophilicity of the benzoyl chloride carbonyl group is sufficient to

permit nucleophilic attack by the pyridine solvent (or imidazole) as shown in **Scheme 39**. This is followed with nucleophilic substitution of the aromatic nitrogen species by secondary alcohol groups on the sugar ring. Subsequent deprotonation of the oxygen atom by the basic solvent completes the reaction scheme.

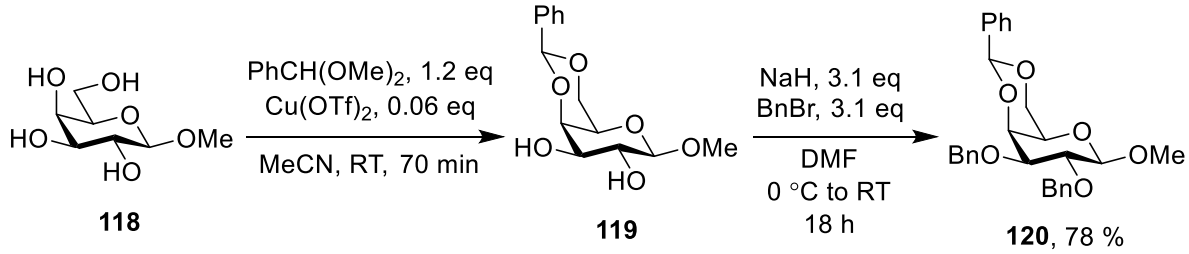


Scheme 39. *Reaction mechanism for esterification of saccharide alcohol groups.*

Quenching excess benzoyl chloride using methanol followed by an aqueous workup affords fully protected glycoside **117**. Deprotection of the silyl ether protecting group can be performed using Brønsted acid catalysed hydrolysis. In this case, stirring in a mixture of THF, water and trifluoroacetic acid was sufficient to fully deprotect the C-6 silyl ether group. Column chromatography gave primary glycosyl acceptor **74** in 67 % yield over three steps.

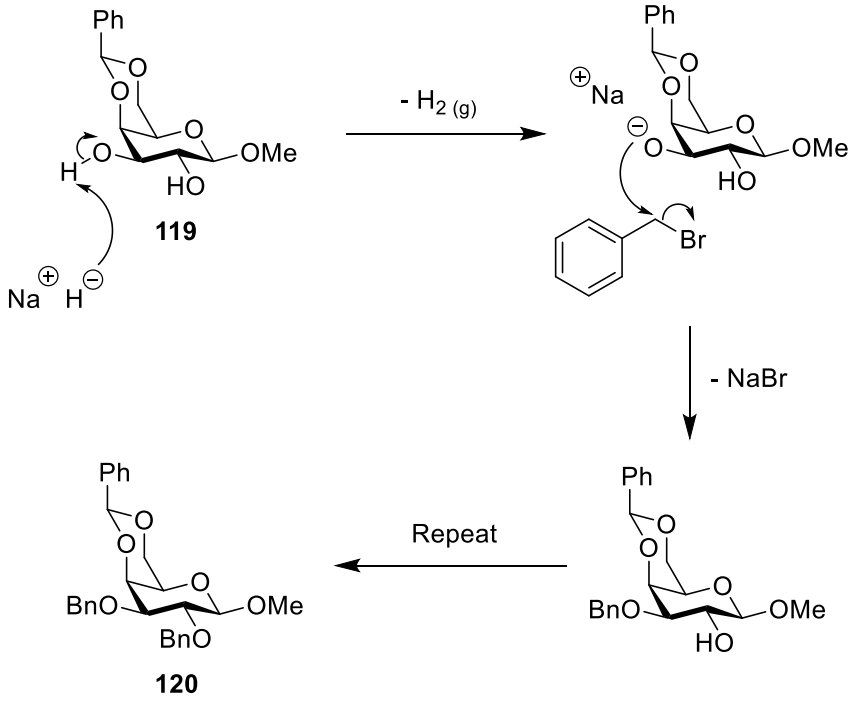
Benzylidene **120** is a useful intermediate as it is a common precursor to a number of different glycosyl acceptors dependent upon the deprotection strategy. Its synthesis begins with methyl β-D-galactopyranoside **118** (**Scheme 40**). Following the catalytic benzylidene acetal protection reported by Galan,[152] the starting material was dissolved in anhydrous acetonitrile followed by addition of copper^{II} triflate and benzaldehyde dimethyl acetal. Sonication for 70 min, deactivation of the copper catalyst with triethylamine and column

chromatography gave intermediate benzylidene **119**. In the benzylation step, saccharide **119** is dissolved in anhydrous DMF, to which sodium hydride and benzyl bromide is added.



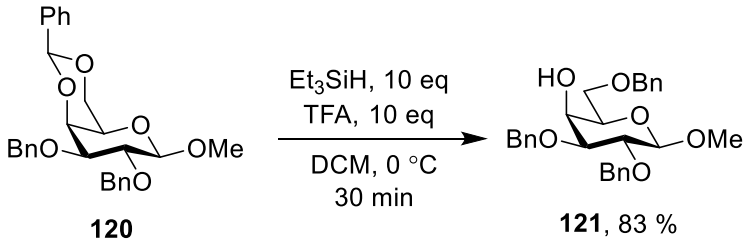
Scheme 40. *Synthetic route from methyl β-D-galactopyranoside* ***118*** *to methyl 2,3-di-O-benzyl-4,6-O-benzylidene-β-D-galactopyranoside* ***120****.*

The strong base sodium hydride is needed to increase the nucleophilicity of the free alcohol groups to allow nucleophilic substitution of the bromine atom in benzyl bromide (**Scheme 41**). Sodium hydride abstracts the alcoholic proton leaving the sodium alkoxide and liberating gaseous hydrogen, the formation of which is both enthalpically and entropically favourable. This more reactive alkoxide species is then able to attack benzyl bromide, thus installing the benzyl protecting group on the alcohol and producing sodium bromide as a side product. After a methanol quench of excess sodium hydride, aqueous workup and column purification fully protected benzylidene **120** was obtained in 78 % yield over two steps.



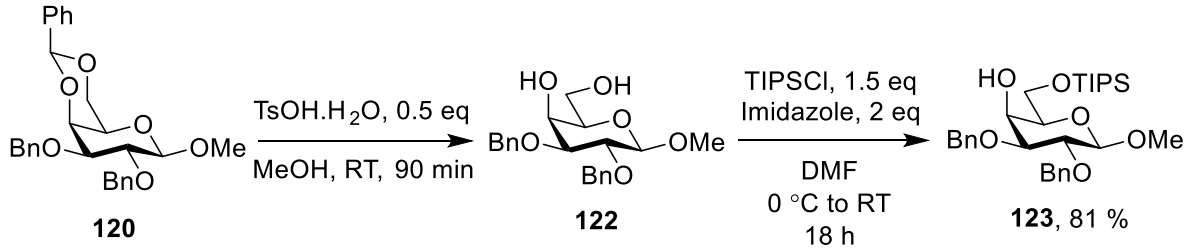
Scheme 41. *Reaction pathway for alcohol deprotonation and attack of benzyl bromide.*

Galactoside **121** can be prepared from benzylidene **120** through regioselective hydride delivery using triethylsilane and trifluoroacetic acid (**Scheme 42**). The underlying rationale for this regioselectivity is not well understood and is susceptible to changes in reagent and solvent. Nonetheless, when the reported conditions[153] are replicated, excellent regiochemistry can be achieved in hydride delivery, allowing preparation of product **121** in 83 % yield.



Scheme 42. *Regioselective reductive ring opening of the benzylidene acetal in **120** affords galactoside **121**.*

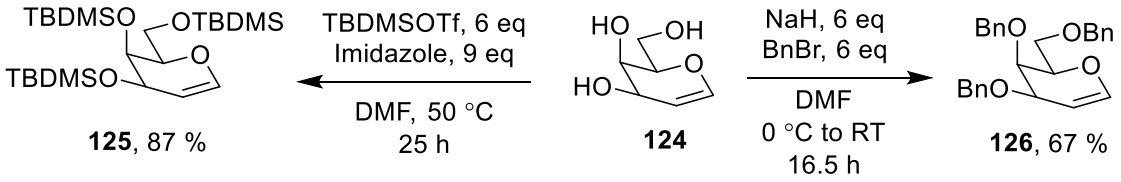
Alternatively, **Scheme 43** shows how acid catalysed cleavage of galactopyranoside **120** with methanol and *p*-toluenesulfonic acid monohydrate affords diol **122**. Treatment of **122** with TIPSCl and imidazole in anhydrous DMF as described previously afforded galactoside **123**.



Scheme 43. *Synthetic route from benzylidene **120** to galactoside **123**.*

4.1.2. **Glycal Donor Syntheses**

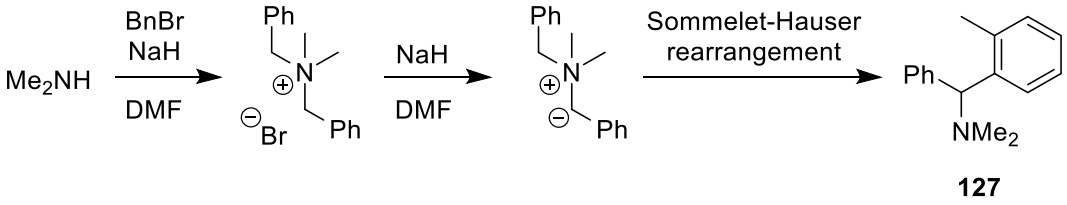
In addition to the synthesis of a range of differentially protected primary and secondary glycosyl acceptors, some glycal donors were also synthesised. For example, beginning from D-galactal **124** persilylation or perbenzylation can be performed in a single step to afford fully protected donors (**Scheme 44**). In this manner *tert*-butyldimethylsilyl ether protected galactal **125** can be prepared in 87 % yield, whilst benzyl ether protected donor **126** can be prepared in 67 % yield.



Scheme 44. *D-Galactal **124** may be protected in a single step to give silyl ether protected donor **125** or benzyl ether protected donor **126** in good yield.*

An important consideration when preparing glycosyl donors and acceptors is ensuring high purity of the final compound. Impurities, even in small quantities, are capable of poisoning catalysts and retarding glycosylation progress. In the case of donor **126**, a study published during my PhD studies showed that when benzylation reactions using benzyl bromide and sodium hydride are performed using DMF as a solvent, the tertiary amine **127** is

formed.[154] The amine **127** is formed from decomposition of DMF into carbon monoxide and dimethylamine and subsequent reaction of the dimethylamine, culminating in a Sommelet-Hauser rearrangement to form **127** as shown in **Scheme 45**. This impurity elutes during flash chromatography at the same time as protected galactal **126** and is thus difficult to separate from the product by this method. The authors showed that amine **127** acts as a poison for thiourea organocatalysts such as **86** and prevents glycosylation from occurring. However, an aqueous acidic wash during workup protonates amine **127**, extracting it into the aqueous phase whilst the glycosyl donor remains in the organic phase. Indeed, when perbenzylated donor **126** was synthesised, after initial column chromatography ^{1}H NMR spectroscopy showed the presence of amine **127**. To remove this, the impure compound was dissolved in hexane and washed with 1 M HCl $_{(aq.)}$, $NaHCO_{3\ (sat.\ aq.)}$ and water. Following drying over magnesium sulfate and removal of solvent, ^{1}H NMR spectroscopy revealed that amine **127** had been completely removed, leaving the desired compound in high purity.



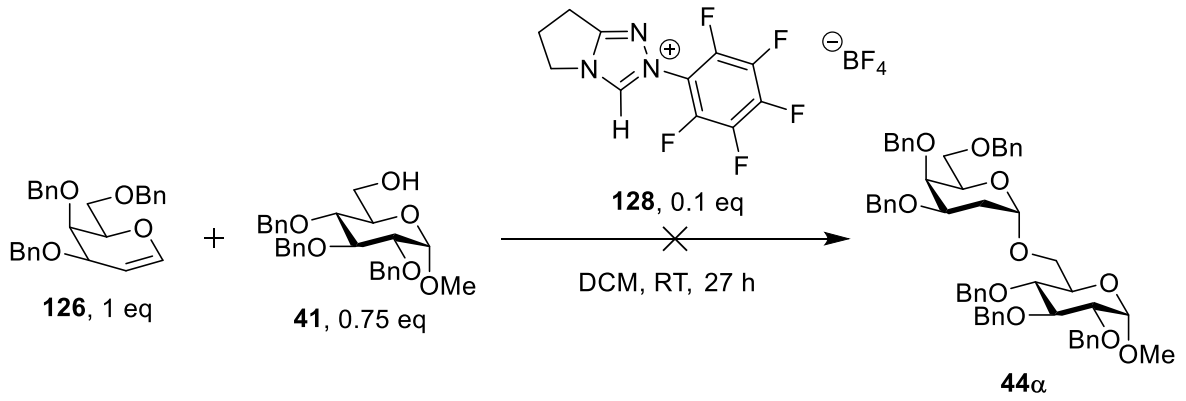
127

Scheme 45. *Dimethylamine, formed from decomposition of DMF, undergoes reaction with benzyl bromide and sodium hydride to form tertiary amine* ***127****.*[154]

4.2. Cooperative Organocatalytic Glycosylation

4.2.1. Experiments Using an Organocatalytic Triazolium Salt

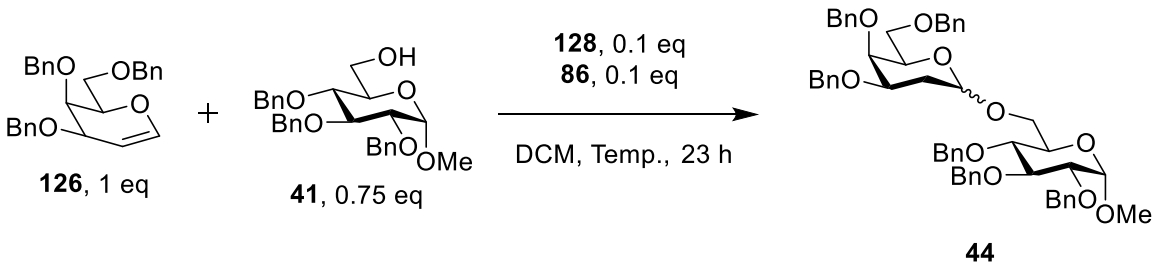
Experiments using triazolium salt **128** were performed in an attempt to organocatalytically synthesise 2-deoxyglycosides from glycals. It is known that salt **128** can be deprotonated to form an *N*-heterocyclic carbene, and it was hypothesised that the salt, with its labile proton, may be able to act as a Brønsted acid. Therefore, a reaction was attempted using donor **126** and acceptor **41** in combination with 0.1 equivalents of salt **128** in order to prepare 2-deoxyglycoside **44α** as shown in **Scheme 46**.



Scheme 46. *Attempted use of triazolium salt **128** as a Brønsted acid organocatalyst.*

Unfortunately, no product was observed even after 27 hours of reaction time. In order to probe the synergistic effects of salt **128** and thiourea **86** on the glycosylation, several experiments were performed as shown in **Table 1**. In entry 1 0.1 eq of thiourea **86** in combination with 0.1 eq of triazolium salt **128** furnished product **44** in 35 % conversion and α:β ratio of 4:1 as determined by ^{1}H NMR spectroscopy. Increasing the temperature from RT to reflux as in entry 2 gives better selectivity but with slightly decreased yield. Finally, in entry 3, thiourea **86** is used as the sole organocatalyst, giving product **44α** in 59 % conversion.

Table 1. *Results from combination of triazolium salt **128** and thiourea **86** as dual organocatalysts.*



Entry	Temperature	NMR Yield of Product 44 (%)	α:β
1	RT	35	4:1
2	Reflux	28	α
3[a]	Reflux	59	α

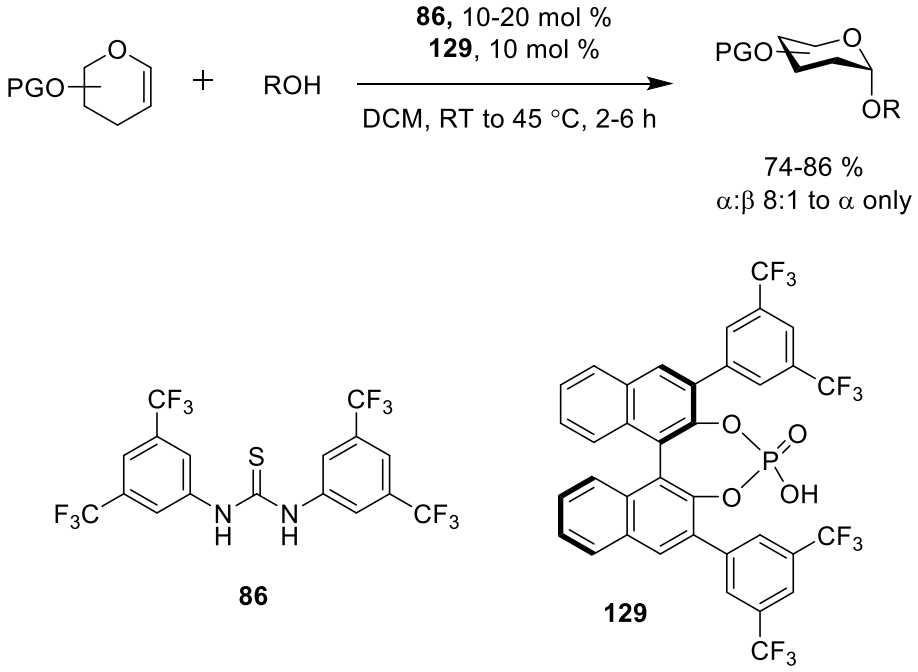
[a]Reaction performed with no triazolium salt **128**.

The results clearly demonstrate that salt **128** is not beneficial to the glycosylation reaction, giving worse results than when thiourea **86** is used alone. This may be rationalised

by considering the pK_a values of the organocatalysts. Thiourea **86** has a pK_a in DMSO of 8.5 ± 0.1[155] whereas triazolium salt **128** is many orders of magnitude less acidic, with a pK_a of 16.5 in aqueous solution.[156] The relative lack of acidity of salt **128** is unlikely to be able to activate the glycal donor for glycosylation and the absence of product formation when **128** was used as an organocatalyst as in **Scheme 46** supports this hypothesis.

4.2.2. **Methanol-d_4 Mechanistic Experiment**

As the project continued, a colleague in the lab, Dr. Carlos Palo-Nieto, identified a cooperative Brønsted acid-thiourea organocatalysed glycosylation protocol as shown in **Scheme 47**.

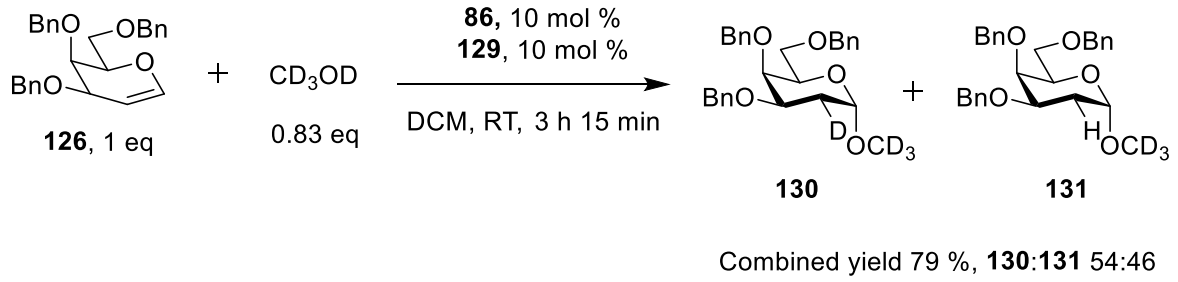


Scheme 47. *α-selective glycosylation of glycals using a cooperative thiourea-Brønsted acid organocatalytic approach.*

By utilising thiourea **86** in addition to chiral BINOL derived phosphoric acid **129**, the glycosylation of several different glycosyl donors, including galactals, glucals and rhamnals, was accomplished with excellent yields and generally >30:1 α stereoselectivities in 2-6 hours at RT or 45 °C. It is noteworthy that the chirality of the acid is able to influence stereoselectivity in the glycosyl product. Acid **129**, the R enantiomer, was used to glycosylate

donor **126** with acceptor **41**, giving the product **44** in >30:1 α:β ratio. However, when the S enantiomer of **129** is used, the stereoselectivity drops to 7:1 α:β. Furthermore, the acceleration of reaction rate by using acid **129** in conjunction with thiourea **86** relative to using thiourea **86** alone is supported by considering the pK_a of the acid, which was calculated as 2.63 ± 0.08 in DMSO.[157] The greater acidity of acid **129** relative to thiourea **86** will activate the glycal donor more rapidly than using **86** as the sole organocatalyst.

I performed a reaction using donor **126** with methanol-d_4 as the glycosyl acceptor in order to help elucidate the mechanism of the reaction. The reaction is shown in **Scheme 48**. The reaction used fully anhydrous conditions; the donor and organocatalysts were dried under vacuum for 16 h in flame-dried glassware prior to the reaction, whilst the methanol-d_4 was dried using activated 3Å molecular sieves prior to use. The reaction gave two products which differ only in the isotope present in the equatorial position at C-2. In compound **130**, the equatorial C-2 substituent is a deuterium atom, whilst in **131** this atom is protium. In both compounds the OCD_3 group has complete α configuration, with no β anomer present. ^{1}H NMR spectroscopy shows that approximately 54 % of the product is fully deuterated **130**, whilst 46 % is protium substituted **131**.



Scheme 48. *Experiment performed using deuterated methanol-d_4 as glycosyl acceptor gave a mixture of two isotopically labelled products **130** and **131**.*

Several important insights can be gleaned from the reaction results. Firstly, no product is present with an axially substituted C-2 deuterium atom. This would suggest that deuteron or proton addition to the glycal occurs on the bottom α face selectively (red path in **Figure 16**), since only this attack orientation would lead to the equatorial deuterium seen in **130**. Conversely, the blue path in **Figure 16** does not appear to occur. This implies a *syn* addition of CD_3OD across the glycal double bond.

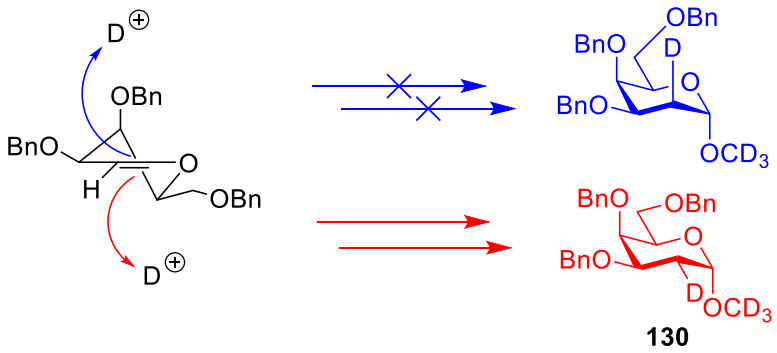


Figure 16. *The two potential orientations for deuteron addition to galactal donor* ***126****. The red path with addition to the "bottom" α face leads to observed product* ***130****, whilst the blue path with addition to the "top" β face leads to an unobserved product with an axially configured C-2 deuterium atom.*

Furthermore, the mixture of **130** and **131** in the observed ratios requires some thought. The equatorial deuterium atom in **130** must come from the alcoholic deuteron in CD_3OD, however, close to half of the total product (product **131**) has two protium atoms bonded to C-2. This must be as a result of either, or both, of the following: i) proton donation to the glycal from molecules other than CD_3OD, or ii) H/D exchange between CD_3OD and the labile protons of other molecules in the reaction mixture prior to glycal activation. In either case, the obviously labile protons present in the reaction mixture are the acidic proton from acid **129** and the two NH protons in thiourea **86** (**Figure 17**). If all these protons were incorporated into the product(s) of the reaction in place of methanol-d_4 deuterons, we would expect to see a product ratio of approximately **130**:**131** = 64:36 based on the stoichiometry of the reaction. However, 46 % of the product mixture is **131**, significantly more than 36 %. The only conclusion to account for this result is that other protons in the reaction mixture are labile enough to depart from their parent molecule and be incorporated in product **131**, at least to some extent. No other hydrogen atoms present in the molecules of the solvent or the reactants ought to be labile enough to be abstracted as protons by any base within the reaction mixture. Therefore, the additional protons incorporated into **131** must have come from some adventitious compound that was unintentionally added to the reaction vessel. The most likely identity of this compound is water, as complete removal of water from the reactants may be very difficult to achieve, even with extensive vacuum drying. This

adventitious water as a proton source may help to explain the observed product distribution ratio.

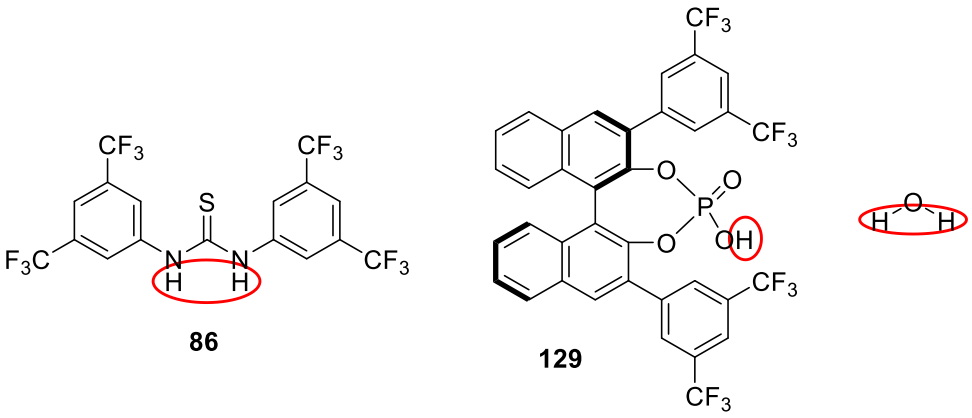
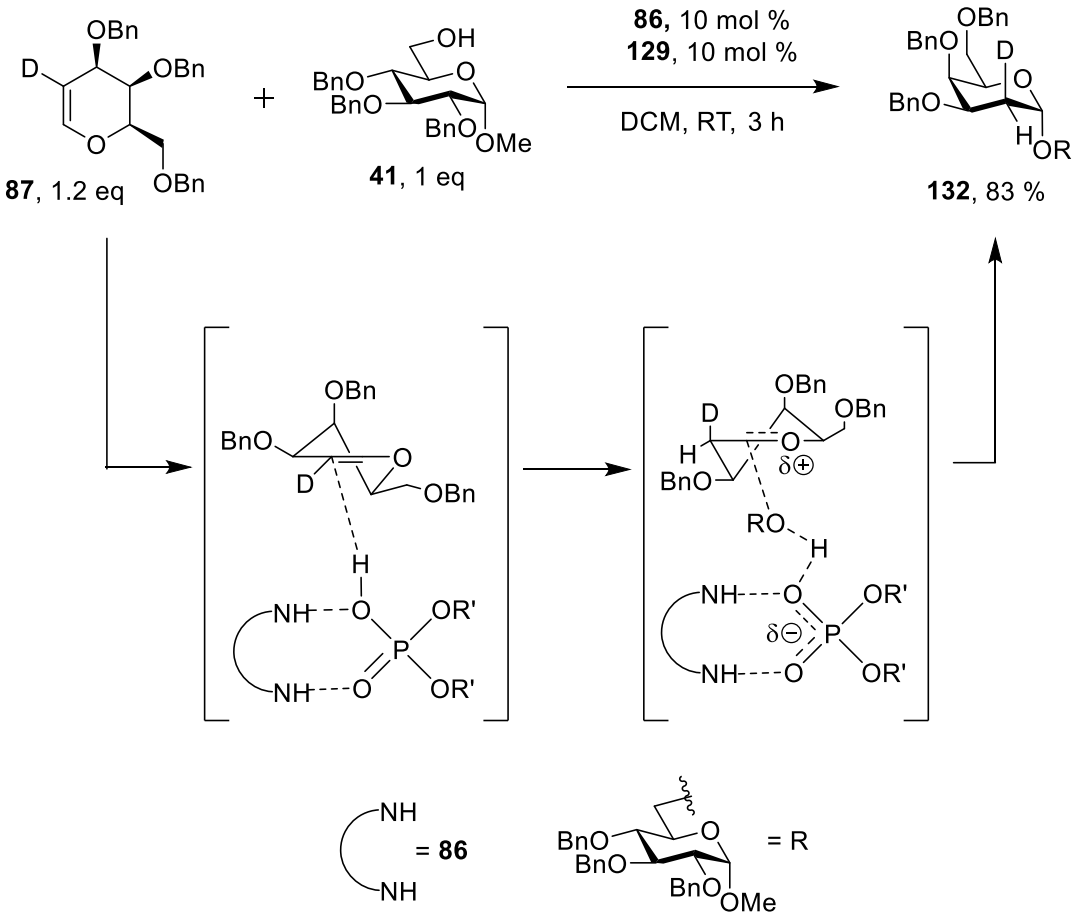


Figure 17. *Probable sources of labile protons within the reaction mixture for the reaction shown in* ***Scheme 48****.*

This reaction gives mechanistic data consistent with the mechanism proposed in the publication for this work,[149] shown in **Scheme 49**, in which deuterated donor **87** was reacted with model acceptor **41** to give product **132** with an axial deuterium atom at C-2. The formation of **132** selectively further supports the hypothesis of a *syn* addition of the glycosyl acceptor alcohol across the "bottom" α face of the glycal donor. Hence, the proposed mechanism involves increasing the acidity of acid **129** through hydrogen bonding with thiourea **86** to give a thiourea-acid complex that delivers a proton to the less hindered face of the glycal donor. The short-lived oxocarbenium ion formed is then trapped by the nucleophilic alcohol acceptor, concomitantly regenerating the acid organocatalyst. However, the results from the reaction using methanol-d_4 as acceptor, as well as further work by Pápai[158] and McGarrigle[159] suggest that the propensity for thiourea **86** to act as a Brønsted acid itself, as opposed to a dual hydrogen bond donor, has been underestimated. As such, the limited role of thiourea **86** solely as a hydrogen bond donor in the proposed mechanism seems unlikely, a more plausible mechanism may involve a complex interplay between thiourea Brønsted acidity and hydrogen bonding.



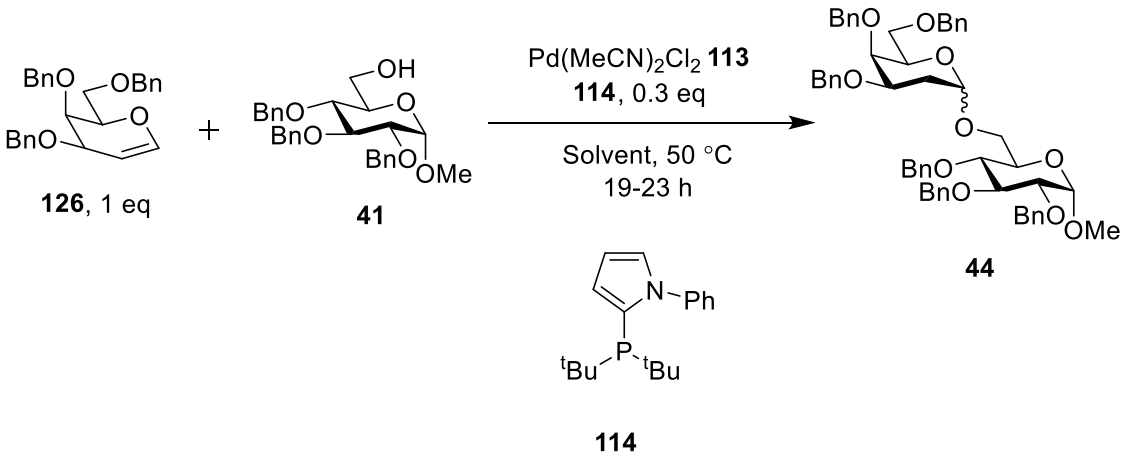
Scheme 49. *Mechanism proposed for the synergistic organocatalytic synthesis of 2-deoxyglycosides using thiourea* ***86*** *and chiral phosphoric acid* ***129****.*

4.3. **Palladium Catalysed Glycosylation**

4.3.1. **Optimisation of Palladium Catalysed Glycosylation**

Prior work in the research group had identified the active $Pd(MeCN)_2Cl_2$ **113** catalyst and ligand **114** described in the project aims. The project was then continued by myself in collaboration with colleagues working in the lab, primarily Dr. Abhijit Sau. The model reaction used to investigate this catalytic reaction is shown in **Scheme 50**. The chosen donor tri-*O*-benzyl-D-galactal **126** and acceptor methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **41** were selected for a number of reasons. Firstly, the benzyl protecting group is generally cleaved to liberate the free alcohol by heterogeneous catalytic hydrogenation using hydrogen gas with solid palladium dispersed on a charcoal support. The benzyl group is, however, stable to most

conditions likely to be experienced during the reaction, including acidic and basic media. Furthermore, the benzyloxide group is a poor leaving group. This helps to discourage the undesired Ferrier rearrangement which requires departure of a C-3 leaving group. Secondly, the acceptor is a primary alcohol. Primary glycosyl acceptors tend to exhibit greater reactivity than secondary acceptors on the basis of less steric hindrance in the transition states of steps in the reaction pathway. Moreover, galactal donors are known to generally display greater reactivity than their glucal counterparts. This heightened reactivity enables identification of conditions most conducive to rapid, selective reaction. Finally, the methoxy group present in the acceptor and disaccharide product(s) offers a useful handle for analysis of the crude reaction mixture by ^{1}H NMR spectroscopy, since the CH_3 peaks for the acceptor and both anomers do not overlap in the ^{1}H NMR spectrum and hence acceptor conversion to product can be calculated based on integration of the peaks.



Scheme 50. *Model reaction for optimisation of palladium catalysed synthesis of 2-deoxyglycosides.*

In order to assess the success of a given set of reaction conditions for the catalytic system in question, two basic metrics should be considered. Firstly, conversion of limiting reagent glycosyl acceptor **41** to the desired 2-deoxy-disaccharide product, with minimisation of side product formation. Secondly, the anomeric α:β ratio of disaccharide product. Ideally only one anomer would be formed. **Table 2** summarises the reactions performed as part of the optimisation of reaction conditions, following the general reaction profile described in **Scheme 50**.

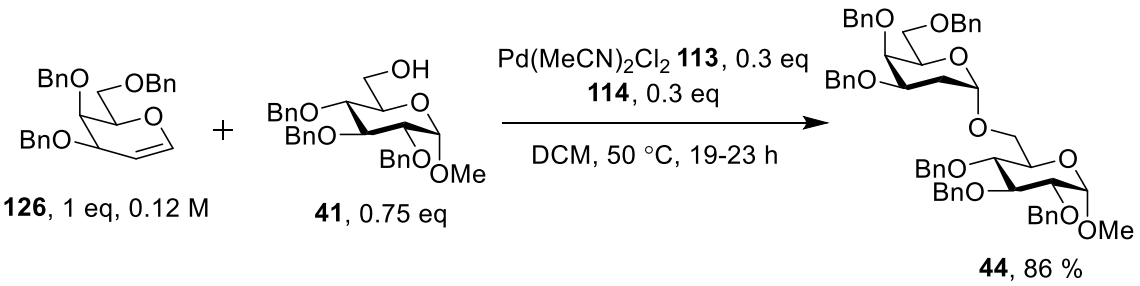
Table 2. *Optimisation experiments performed and conditions used in each. All reactions were performed using 50 mg (1 eq) of glycosyl donor* ***126****.*

Entry	Acceptor 41 eq	$Pd(MeCN)_2Cl_2$ eq	Solvent	Conversion of 41 to 44 (%)	α:β
1	0.5	1.0	MeCN 2 mL	27	2.9:1
2	0.5	**0.5**	MeCN 2 mL	79	6.2:1
3	**0.75**	**0.4**	**DCM** 2 mL	28	2.5:1
4	0.75	**0.3**	DCM 2 mL	93	α only
5[a]	0.75	0.3	DCM 2 mL	53	7.8:1
6	0.75	0.3	DCM **1 mL**	99	α only

[a]Reaction performed at RT.

In entry 1, when a stoichiometric amount of palladium complex is used, conversion to the product and stereoselectivity are both very poor, whilst entry 2 shows that decreasing the amount of palladium complex **113** to 0.5 eq gives a dramatic boost to conversion from 27 % to 79 %. At this point in the project, a colleague's experiments showed that DCM was a superior solvent for the glycosylation than MeCN, and thus further experiments used DCM as the solvent (refer to the publication accompanying this work for further information).[150] This is likely due to the ability of the nitrogen atom in MeCN being able to complex the palladium atom, tempering its Lewis acidity, whereas DCM has no moiety that can effectively chelate palladium. Comparing entries 3 and 4 shows that in DCM, even a slight excess of palladium complex **113** relative to the 0.3 eq of ligand **114** used is extremely detrimental to product formation. However, in entry 4 where equivalent amounts of complex **113** and ligand **114** are used, excellent conversion to product as well as complete α stereoselectivity are seen. Reaction temperature is important for the reaction, as shown in entry **5**, which shows that the reaction performed at RT as opposed to 50 °C causes a large drop in conversion and selectivity. Finally, in entry 6, the volume of solvent is decreased such that the concentration of the reaction goes from 0.06 M in donor in entry 4 to 0.12 M in donor in entry 6. In this case, 99 % conversion to product is seen, with complete α selectivity.

The conditions found in entry 6 represent the optimised conditions for the reaction, shown in **Scheme 51**, allowing the preparation of disaccharide **44** as the α anomer in 86 % isolated yield. These conditions were carried forward to assess the substrate tolerance of the reaction. My role in the project was to probe the tolerance of the method for a library of glycosyl acceptors.



Scheme 51. *Optimised conditions for palladium catalysed 2-deoxyglycoside synthesis from glycals. Disaccharide* ***44*** *was isolated in 86 % yield as the α anomer.*

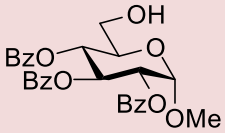
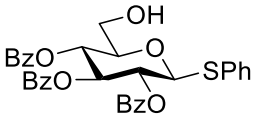
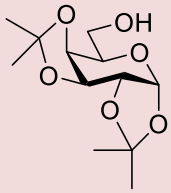
4.3.2. **Acceptor Scope**

4.3.2.1. Primary Saccharide Glycosyl Acceptors

Acceptor alcohols were screened according to the optimised conditions discussed above, with the model galactal donor **126** used in each case. The glycosyl acceptors tested in the catalytic system are shown in **Table 3**. The reaction of glucoside **74** (entry 1) proceeded smoothly to give very high yields of desired product, with excellent α selectivity. In entry 2, the thioglycoside acceptor **133** was used. The thiophenyl moiety at the anomeric position present in this acceptor offers the potential for further glycosylation reactions on the disaccharide product to give oligosaccharides. However, the concern for this acceptor was that the sulfur atom may irreversibly bind to the palladium atom, thus reducing catalytic activity and resulting in a decrease in yield. As expected, the obtained yield of 56 % was somewhat lower than the structurally similar acceptor **74**, whilst the α selectivity observed is also lower. As the project progressed, deuterated reactant studies helped to elucidate the probable mechanism of the reaction. Knowledge of the reaction mechanism allowed repetition of the experiment in entry 2 by a colleague, but by altering the order of addition of

reagents a yield of 84 % was obtained, as described below during discussion of the reaction mechanism in section **4.3.3.**

Table 3. *Results from screening of primary saccharide derived alcohols.*

Entry	Acceptor	Time (h)	Conversion of acceptor to product (%)	α:β	Isolated Yield (%)
1	 **74**	18	90	>30:1	82
2	 **133**	21	-	6:1	56 (α only)
3	 **63**	42	64	1.3:1	36 (α only)

Finally, in entry 3 the isopropylidene protected galactose acceptor **63** was screened. In this reaction, very poor α selectivity was observed, as well as low conversion to the desired product. This may be due to the steric hindrance caused by the 3,4 isopropylidene group. For benzyl and benzoyl protecting groups the large phenyl ring can be positioned far from the sugar ring in space by using the OCH_2 or OCO groups as a "hinge", whereas the rigid and bulky CMe_2 group of isopropylidene is held much closer in space to the sugar ring, possibly affording greater steric hindrance during steps along the reaction pathway. Moreover, acceptor **63** is the only primary galactose derived acceptor screened. It is likely that the axial (C-4)-O configuration also contributes to steric clash not experienced by the equatorial (C-4)-O configuration seen in glucose derived acceptors.

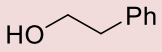
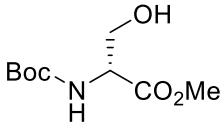
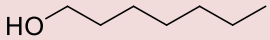
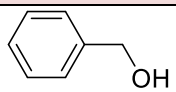
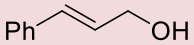
A much longer reaction time of 42 h was required to achieve a reasonable conversion of acceptor **63**, determined to be approximately 64 % from ^{1}H NMR spectroscopy. Generally, separation of α and β anomers by column chromatography is difficult or impossible, but in

this case the anomers were successfully separated to give a pure sample of the α anomer in 36 % yield.

4.3.2.2. General Primary Alcohol Acceptors

To evaluate the applicability of the protocol for general couplings of alcohols to glycal donors, a number of general primary alcohols were screened. The results of these experiments are shown in **Table 4**.

Table 4. *Results from screening of primary general alcohols.*

Entry	Acceptor	Time (h)	Conversion of acceptor to product (%)	α:β	Isolated Yield (%)
1	 **134**	18	-	5:1	69 (α only)
2	 **135**	18	95	α only	88
3	 **136**	18.5	-	9:1	63
4	 **137**	17	-	>30:1	96
5	 **138**	23	-	α only	66

In general, very pleasing α selectivity was seen, with two examples showing only α product formed. Furthermore, the 2-deoxy-saccharide products were obtained in good to excellent yields, with all reaction times under 24 h. It is worth noting that for many of these alcohols, it is difficult to determine a quantitative conversion from the crude ^{1}H NMR spectrum, as there is no NMR "handle" signal to monitor that doesn't overlap between reactant and product(s). In many cases however, it is possible to qualitatively determine whether significant reaction has occurred, using for example the diagnostic peaks corresponding to the axial and equatorial C-2 protons on the 2-deoxysaccharide ring.

A complication during some of the syntheses was the purification. The presence of phosphine ligand **114** was detected through two principal methods: TLC and ^{1}H NMR spectroscopy. TLC plates were usually stained by treatment with a solution of sulfuric acid in ethanol, then charred using a heat gun to enable visualisation of compounds. This staining method works well for carbohydrates, whether protected or unprotected, and sometimes shows other types of compound on the TLC plate depending upon the functional groups featured on the compound in question.

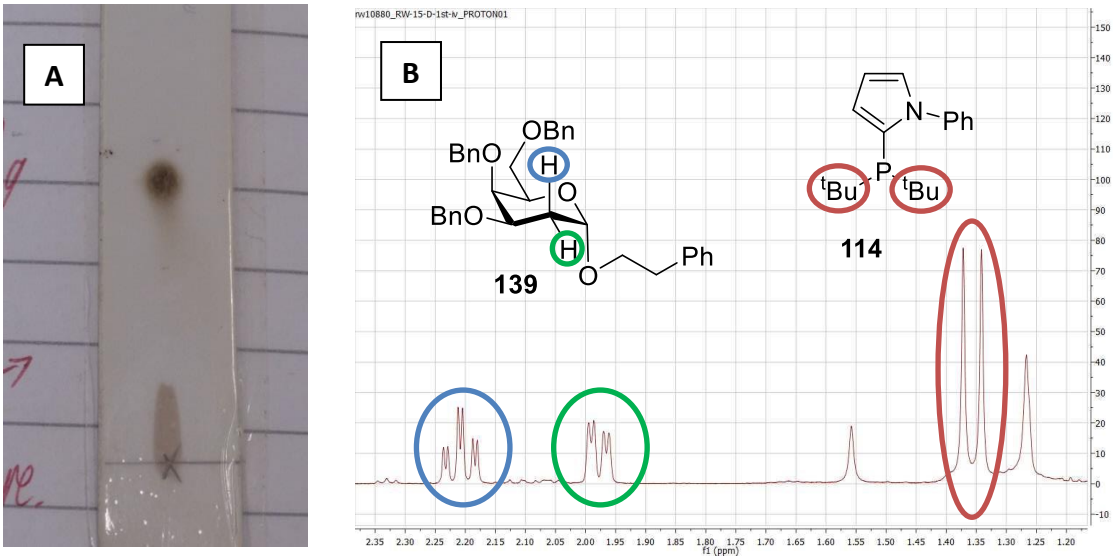


Figure 18. A. *TLC plate from the reaction to form 2-deoxyglycoside* ***139*** *as shown in Entry 1 of* ***Table 4****.* ***B.*** *Section of the ^{1}H NMR spectrum for 2-deoxyglycoside* ***139*** *showing H-2 peaks and ^{t}Bu peaks from ligand* ***114****.*

Figure 18 A shows a typical TLC plate (Hexane:EtOAc 7:3) for the reaction between model glycal donor **126** and phenethyl alcohol **134** after an initial column purification (α and β anomers were successfully separated for this compound). The product glycoside **139** is seen as the darker upper spot at $R_f \approx 0.6$, whilst ligand **114** can be seen as the streak covering $R_f \approx$ 0-0.16. **Figure 18 B** shows a section from the ^{1}H NMR spectrum of **139**. The doublet of triplets and doublet of doublets on the left correspond to protons H-2a and H-2b on the sugar ring. The ligand impurity can be seen as the two large peaks ringed in red, corresponding to the $C(CH_3)_3$ signals from the two *tert*-butyl groups, whilst the remaining peaks seen in this section of the spectrum arise from impurities in the solvent.

Despite the apparent high polarity of the ligand suggested by the silica TLC plate, when performing column chromatography the ligand tends to elute from the column at around the same time as other products with an R_f of ≈ 0.6 (Hexane:EtOAc 7:3); including the reaction products from entries 1, 4 and 5 from **Table 4**. Even HPLC was found to be insufficient to separate the mixture. After experimenting with trituration purification unsuccessfully, it was found that running a very long silica column at a shallow solvent gradient can at least partially separate the mixture to give a product of acceptable purity. **Figure 19** displays a section of another ^{1}H NMR spectrum of 2-deoxyglycoside **139** that has undergone a lengthy column purification. Integration of saccharide and ligand peaks reveals a ratio of 1H(saccharide):9H(ligand) = 3.37:1, corresponding to ≈ 97 % purity of the product.

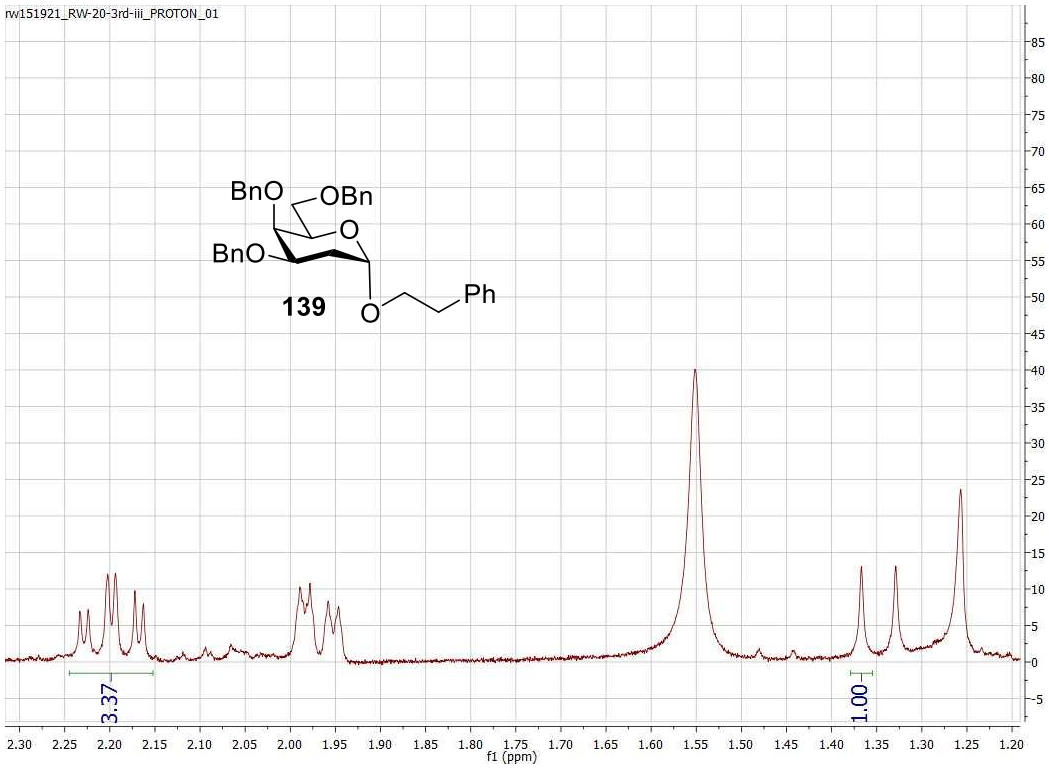


Figure 19. *Section of the ^{1}H NMR spectrum for 2-deoxy-saccharide **139** with greater purity than that shown in **Figure 18 B**.*

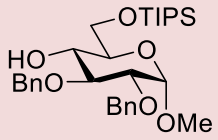
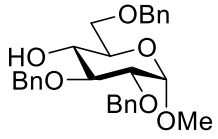
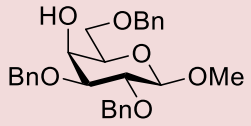
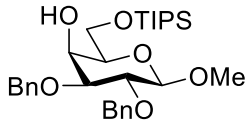
Of course, this purification difficulty only arises for products that have R_f of ≈ 0.6 (Hexane:EtOAc 7:3). For compounds such as the heptyl 2-deoxyglycoside product from entry 3 in **Table 4** (R_f > 0.6) and the protected serine glycoside product from entry 2 (R_f < 0.6), purification proceeds smoothly. A further noteworthy point is that the reaction using

cinnamyl alcohol **138** as a substrate proceeded satisfactorily, indicating that the palladium complex can tolerate the presence of an alkene group in the reaction mixture without its catalytic activity being compromised.

4.3.2.3. Secondary Saccharide Glycosyl Acceptors

With the greater steric hindrance found for a secondary alcohol, reactivity is generally lower than that of primary alcohols. **Table 5** documents the secondary saccharide derived glycosyl acceptors screened for activity in the catalytic glycosylation system.

Table 5. *Results from screening of secondary saccharide derived alcohols.*

Entry	Acceptor	Time (h)	Conversion of acceptor to product (%)	α:β	Isolated Yield (%)
1[a]	 **140**	46	82	>30:1	64
2	 **53**	45	-	4:1	72
3	 **121**	45	-	3.5:1	70
4	 **123**	45	-	-	Complex Mixture

[a]With a subsequent silyl ether removal step using TBAF for purification purposes.

As expected, yields were found to be lower than some of the primary glycosyl acceptors, but certainly not prohibitively low. However, in order to achieve complete reaction, reaction times of around 45 h were required, in contrast to the 18 h generally needed for primary acceptors. Entry 1 reveals that interestingly, despite the bulky C-6 TIPS protecting group present in glycosyl acceptor **140**, good selectivity and a moderate yield were obtained. In entries 2 and 3, although yields are good for the reactions, the major problem is

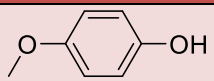
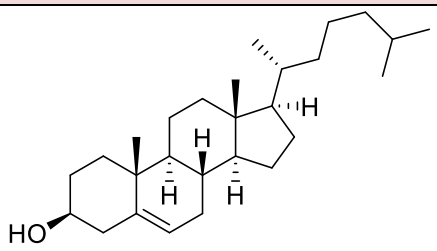
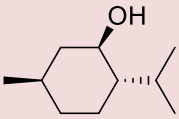
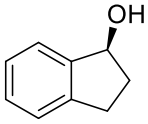
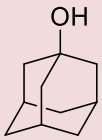
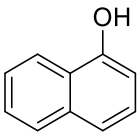
poor stereoselectivity for the 6-*O*-benzyl acceptors **53** and **121**. Finally, in entry 4, the use of galactose derived donor **123** bearing a C-6 TIPS protecting group gave a complex mixture of products from which no desired product could be isolated.

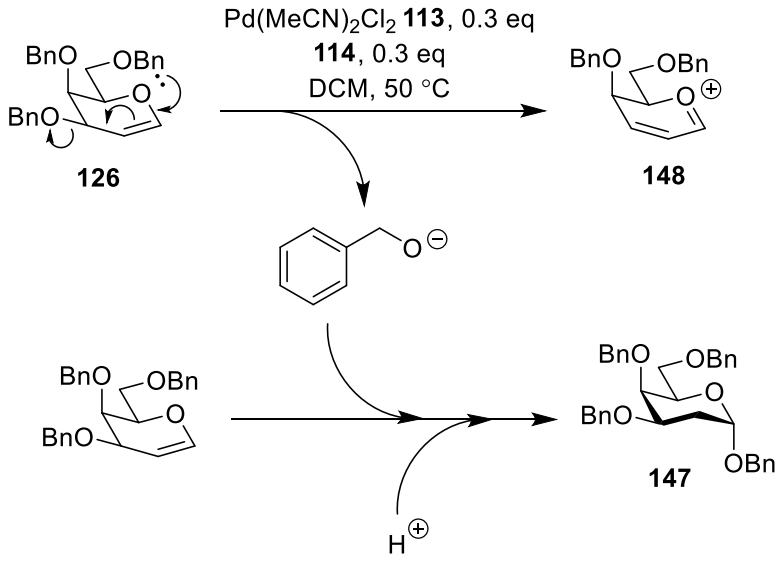
4.3.2.4. General Secondary Alcohol Acceptors

Next, the applicability of the method for a range of aliphatic and aromatic secondary and tertiary alcohols was examined (**Table 6**). Unfortunately, the results obtained were rather unsatisfactory, with difficulties being encountered in both selectivity and yield. Entry 1 shows that the electron rich aromatic glycosyl acceptor *p*-methoxyphenol **141** successfully gave the desired product in moderate yield, albeit with poor selectivity. In entries 2 and 3, acceptors cholesterol **142** and menthol **143** also gave the desired products, but in poor yield. Other glycosyl acceptors tested, including (S)-1-indanol **144**, the tertiary alcohol 1-adamantol **145** and 1-naphthol **146** did not form any of the desired product. Instead, the formation of deoxyglycoside **147** is confirmed by examining the ^{1}H NMR spectra of the crude product mixtures from entries 4, 5 and 6.

2-Deoxyglycoside **147** was intentionally formed as the product of entry 4, **Table 4**, in which benzyl alcohol **137** was used as the glycosyl acceptor. However, in the entries shown in **Table 6**, no benzyl alcohol is added to the reaction mixture. The likely mechanism for the formation of 2-deoxyglycoside **147** is shown in **Scheme 52**. Under the reaction conditions with $Pd(MeCN)_2Cl_2$ **113**, Lewis acid catalysed Ferrier rearrangement of galactal donor **126** may occur to give cation **148** and liberate a benzyloxide anion. This anion may go on to act as a nucleophilic glycosyl acceptor with another molecule of donor **126**, forming the observed product **147**. This reaction pathway only appears to predominate when the reactivity of the desired glycosyl acceptor is low, as is the case in entries 4, 5 and 6 of **Table 6**.

Table 6. *Table displaying results from general secondary and tertiary alcohols screened.*

Entry	Acceptor	Time (h)	Conversion of acceptor to product (%)	α:β	Isolated Yield (%)
1	 **141**	24	-	2.2:1	64
2	 **142**	44	-	6:1	42
3	 **143**	42	-	6:1	30
4	 **144**	46.5	Rearrangement product **147**	-	-
5	 **145**	46.5	Rearrangement product **147**	-	-
6	 **146**	42	Rearrangement product **147**	-	-



Scheme 52. *Ferrier rearrangement of donor **126** liberates a benzyloxide anion, which may go on to glycosylate another molecule of the donor to give 2-deoxyglycoside **147**.*

4.3.3. **Mechanism of Palladium Catalysed Glycosylation**

As the project continued, colleagues in the lab conducted mechanistic experiments, including deuterated reactant NMR spectroscopy studies similar to those described in section **4.2.2.** and a mechanism consistent with the experimental data was proposed (**Figure 20**). For full details of these mechanistic elucidation experiments, please refer to the publication in which this work was reported.[150] It should be noted that no palladium oxidation states are given in the catalytic cycle, as no evidence for metal oxidation state was acquired; therefore any oxidation state assertions would be entirely speculative. However, one might consider that whilst complex **113** featuring palladium(II) is added to the reaction mixture, it is possible that the active catalyst contains palladium(0), with the metal having being reduced from palladium(II) *in situ*.

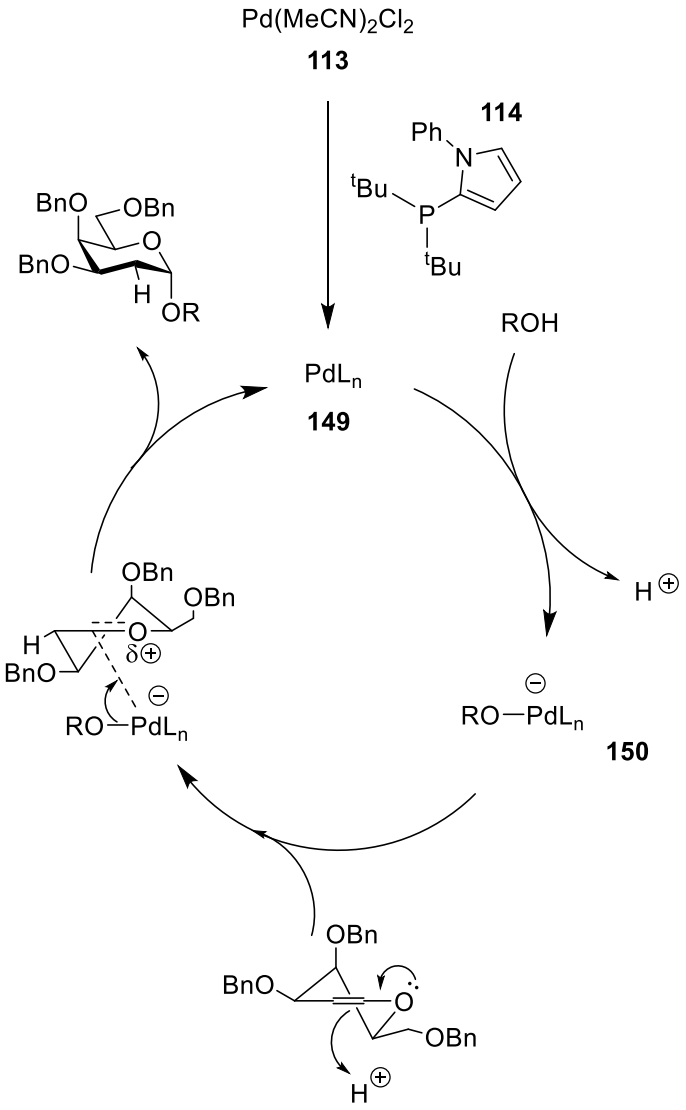


Figure 20. *Proposed catalytic cycle for the palladium catalysed glycosylation of glycals to form 2-deoxyglycosides.*

The cycle begins with addition of the phosphine ligand **114** to the palladium centre to give the active catalyst **149**, the exact nature of which is unknown. This complexation of the palladium metal by ligand **114** serves to attenuate the catalytic activity of the catalyst by decreasing Lewis acidity, thereby minimising side product formation. It also helps to prevent direct coordination of other potential ligands through electronic and steric saturation at the metal centre. The reaction is then thought to proceed, not through palladium coordination to the glycal π bond as one might expect, but rather through addition of the alcohol acceptor to the palladium centre to give an alkoxy-palladium intermediate **150** with concomitant release of a proton. Proton catalysed glycal activation gives a short-lived oxocarbenium ion

that is trapped by intermediate **150** through delivery of the alkoxide to the α face of the sugar ring, regenerating the catalytic palladium species and furnishing the product deoxyglycoside with the newly formed C-2-H and C-1-O bonds *cis* to one another. Note that a hydroalkoxypalladation mechanism, in which formal alcoholic oxidative addition to palladium occurs to form a Pd-H bond, cannot be completely discounted. However, experiments in which bases 1-phenylpyrrole or potassium carbonate were added to the reaction mixture return only starting materials, suggesting that Brønsted acid catalysis features in the mechanism.

With reference to the reaction of thioglycoside acceptor **133** as described in section **4.6.1.**, mechanistic information allows far more complete reaction than the standard procedure followed allows. Active catalyst **149** is preformed by dissolving metal catalyst **113** and phosphine ligand **114** in DCM, preferably with a slight excess of phosphine ligand to ensure the greatest conversion to the active catalyst. A separate solution of donor and thioglycoside acceptor can then be made up and added to the active catalyst solution, beginning the catalytic cycle. This order of addition of reagents means that the palladium is not poisoned by irreversible chelation by the sulfur atom from the acceptor, as the phosphine ligand is already coordinated to the palladium atom prior to contact with the thioglycoside.

4.4. Glycosylation Using Continuous Flow Techniques

The other major facet of my research throughout my PhD aside from identifying and exploring novel catalytic glycosylation systems was the development of continuous flow techniques applied to glycosylations. Firstly, the continuous flow apparatus used will be described, followed by the results of gold(I) catalysed glycosylations in flow. Finally, results from I-Tag supported glycosylation experiments in flow will be reported.

4.4.1. Flow Reactors Used

Two different types of flow reactor were used for experiments for this project. The first approach taken was a reasonably simplistic, yet pragmatic and cheap, coil reactor (**Figure 21**). The reactor was made using PTFE and/or stainless-steel tubing of the type used for HPLC instruments (inner diameter 0.102 cm). Two introductory tubing pieces, each connected to a syringe, met at a T-junction. This T-junction led to a length of reactor tubing, in which the reaction takes place. Solution leaving the reactor tubing may be chemically quenched in a receiving flask. Syringe pumps were used to flow the solution through the reactor at a well-defined flow rate. The coil reactor was primarily used for the gold catalysed glycosylations discussed in section **4.5.**

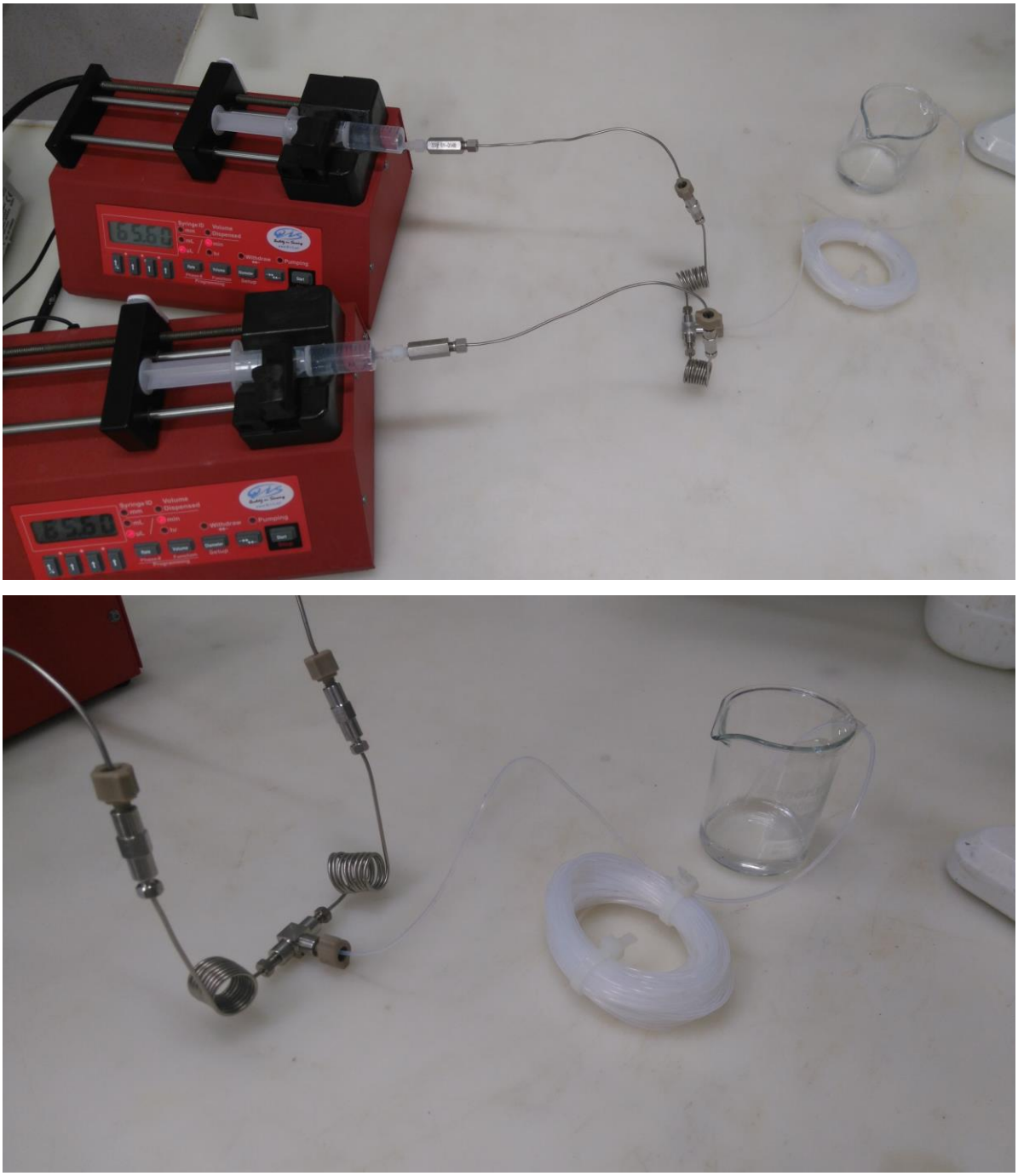


Figure 21. *Coil reactor constructed for flow glycosylation experiments. Here, the introductory tubing pieces are stainless steel, as is the T-junction. These lead to a 5 m length of PTFE reactor tubing, collected into a bundle, which deposits the reaction solution into the beaker.*

The second flow reactor was purchased later into the project from Micronit Microfludics. This was a microreactor setup consisting of a microfluidic borosilicate glass slide with a channel etched into it, for a total internal volume of 18.7 μL (**Figure 22**). The microreactor chip features two inlet ports and a single outlet port, where PTFE tubing can be affixed using ferrules. The PTFE tubing used in this system has a much a smaller internal diameter (0.03 cm) than the coil reactor tubing, in keeping with the smaller dimensions of the channels in the reactor chip. The inlet tubing pieces are connected to syringes containing reactant solutions, whilst the outlet tubing piece leads to a receiving flask. The small dimensions of this microreactor chip maximise the rapid mixing and heat transfer that make flow chemistry advantageous, whilst proving well suited to very short residence times, or to small quantities of material. This reactor chip was used for the glycosylation of I-Tagged sugars as discussed in section **4.6.**

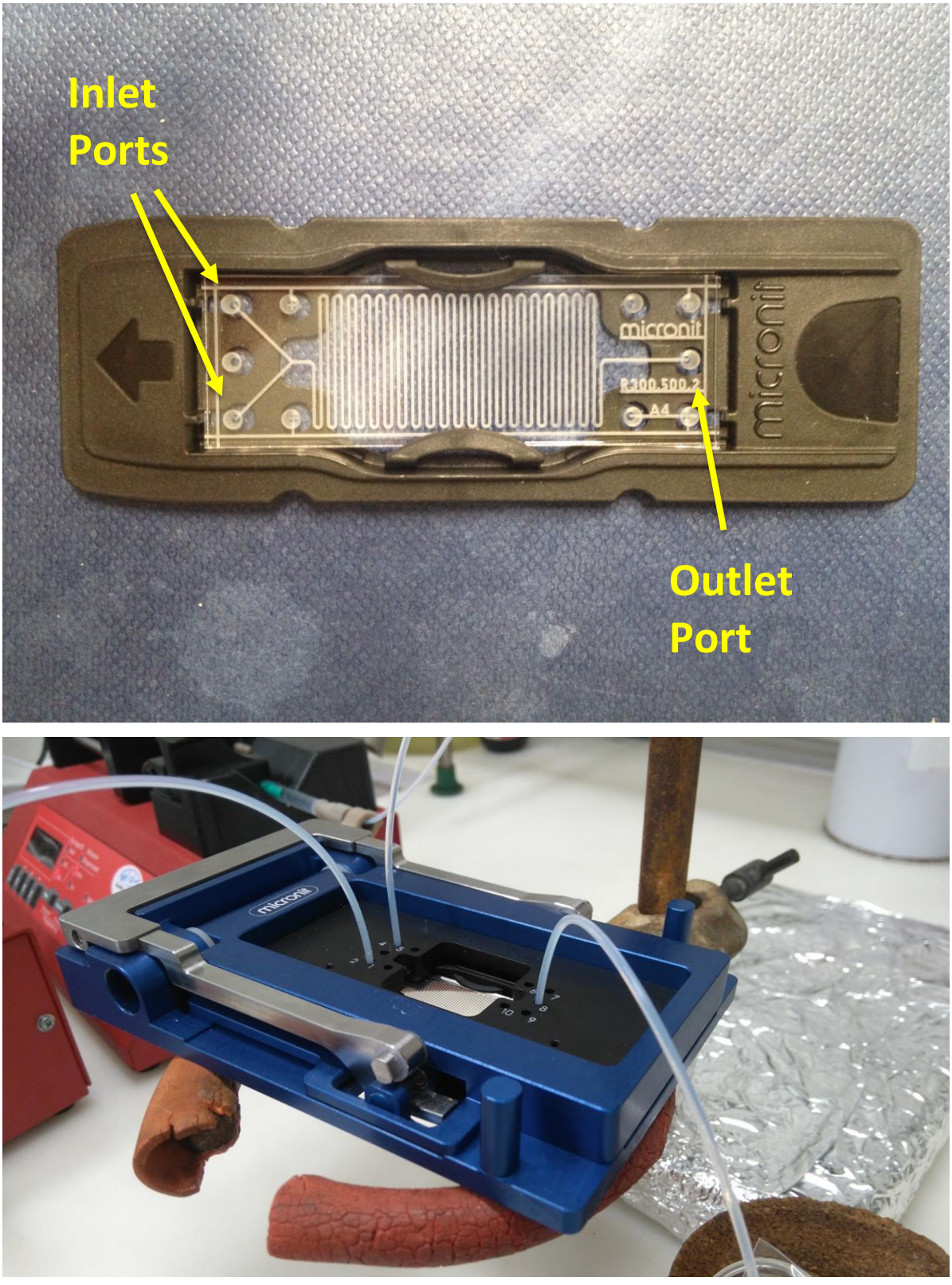


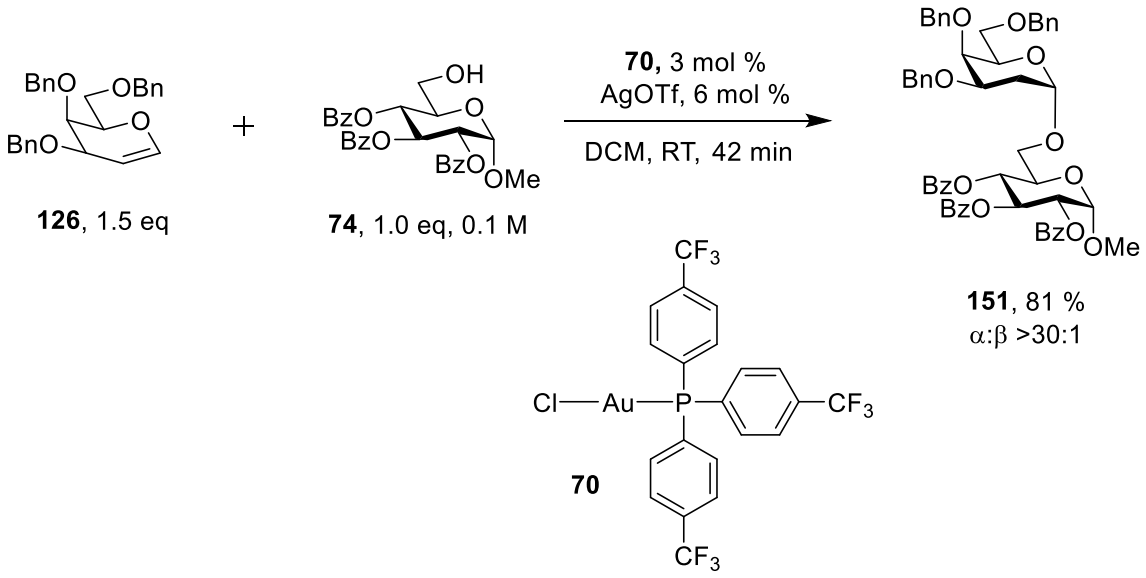
Figure 22. *Microreactor chip used for flow reactions. The top image shows the chip in detail, with the inlet and outlet ports highlighted. The bottom image shows the chip fixed in its cradle, with tubing attached. One of the syringes held in its syringe pump can be seen in the background.*

Several other factors are worth thinking through to clearly design a flow reaction. Firstly, the flow rate for each of the two syringes must be set at half of the desired flow rate for the reaction, since two solution streams are coming together, and the volume of the reactor remains constant. Secondly, the reagent solutions that are made up for injection into the flow reactor must be made up at double the desired concentration for the flow reaction. This is again because two solution streams are joining together and there will be twice as much solvent for the solutes to dissolve in when the reaction occurs. One must also consider some sort of quench to stop the reaction, or the reaction may continue after leaving the flow reactor, invalidating the residence time. In some instances, such as air or moisture sensitive reactions, exposure to air or a reagent grade “wet” solvent may be sufficient to quench the reaction. In other instances, a specific chemical must be added to the receiving flask to halt the reaction.

Fourthly, solid particles that precipitate during the chemical reaction are anathema to continuous flow methods, as solids can block the reaction tubing, preventing solution from flowing. Hence, ensuring all solutes remain dissolved is imperative for successful flow reactions. A final concern is the concentration of solutions that are to be injected into the reactor. This becomes especially relevant when small volumes of solvent are used, and the solution is concentrated. As the solutes dissolve in the solvent, the final volume of the solution may be significantly higher than the volume of solvent added. This means that the final concentration of the solution must be calculated using the observed final volume of the solution. For instance, if 0.4 mL of solvent is added to the desired solutes of known molar amounts, the volume of the resulting solution might be 0.5 mL. Hence, the concentration of solution must be calculated using a volume of 0.5 mL, not the 0.4 mL of solvent added. In practice, this minor difference in solution concentration ought to make little difference to the chemical reaction. However, it does make a difference when percentage yields are calculated, since the theoretical maximum yield is derived from the volume of solution that passes through the flow reactor, which is generally calculated by timing the flow reaction.

4.5. Flow Glycosylations Catalysed by a Gold(I) Catalyst

Investigations into the glycosylation of glycals began following reported batch conditions. Gold catalyst **70**, featuring an appropriate phosphine ligand, is used in combination with silver triflate to catalyse this glycosylation, in which reactions are generally complete in 30-45 min. The model reaction chosen for preliminary screening used benzyl protected galactal donor **126** and primary alcoholic acceptor **74** to give 2-deoxyglycoside **151**. Disaccharide **151** was prepared in batch according to the procedure reported by Palo-Nieto *et al*.[99] as shown in **Scheme 53**.

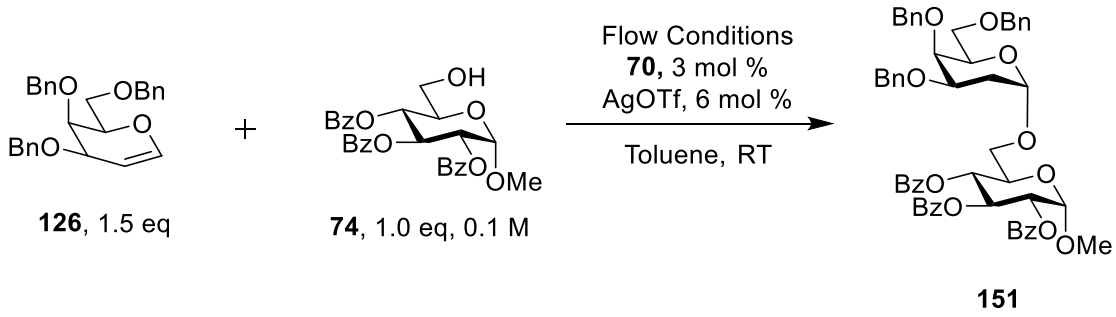


Scheme 53. *Model batch reaction for glycosylation of glycal **126** and glycosyl acceptor **74** catalysed by gold(I) catalyst **70**.*

For the flow reactions, the conditions shown in **Scheme 53** were followed precisely, except for the solvent. DCM is a poor solvent for silver triflate; however, toluene allows dissolution of silver triflate to give a slightly cloudy mixture and does not compromise yield in this reaction. Since ensuring the tubing does not become blocked is very important in flow, toluene was chosen as the reaction solvent. One solution containing the donor and acceptor and another containing gold catalyst **70** and silver triflate were made up and these solutions were flowed directly through the nitrogen-flushed flow reactor. Reactions were quenched using a solution of triethylamine in DCM in the receiving flask. The first flow reaction

performed using this reaction protocol used the coil reactor with a residence time of 45 min and furnished the product disaccharide **151** in 75 % yield and >20:1 α selectivity, coming very close to that achieved in batch. Encouraged by this exciting result, experiments were then undertaken to explore the necessary residence time to allow high yields in this reaction. The results are shown in **Table 7**. All results showed very high (>20:1) selectivity for the α anomer. NMR yield was calculated from the ^{1}H NMR spectrum by comparison of the OCH_3 singlet peaks for acceptor **74** and product **151**.

Table 7. *Results from early flow reactions using the coil reactor and following reaction conditions in* ***Scheme 53****. Residence time for the reaction was varied and yield of product according to ^{1}H NMR spectroscopy was recorded.*



Entry	Residence Time (min)	Conversion of 74 to 151 (%)
1	1	33
2	3	55
3	5	66
4	7.5	80
5	10	89
6	20	85
7	30	80
8	40	82

The results show that under flow conditions, glycosylation can be achieved in high yields in a residence time of just 10 minutes (entry 5). As the time is decreased from 10 minutes, the NMR yield steadily decreases to just 33 % conversion for a 1 minute residence time (entry 1-4), whilst the yield also decreases slowly if the residence time is longer than 10 minutes, as shown in entries 6-8, suggesting a residence time of 10 minutes is the optimum for this reaction. This appeared to be an excellent starting point for further research, however, a repeat reaction of the 10 minute conditions in entry 5 of **Table 7** gave a lower NMR yield of 63 % with no clear explanation as to why. In an effort to use less precious reagent and to increase reproducibility, an alternative method was devised, closely following a literature procedure described by Seeberger and co-workers on a similar gold catalysed glycosylation in flow.[127] In this method, rather than making up large quantities of reaction solutions and flowing them directly through the reactor, the reactor can instead be flushed with anhydrous solvent (toluene), then a small amount of each of the two reaction solutions can be injected into the two introductory tubing pieces. Reconnecting solvent syringes to the reactor and setting the desired flow rate allows the solvent to push the reaction solutions through the reactor. This approach is illustrated more clearly in **Figure 23**.

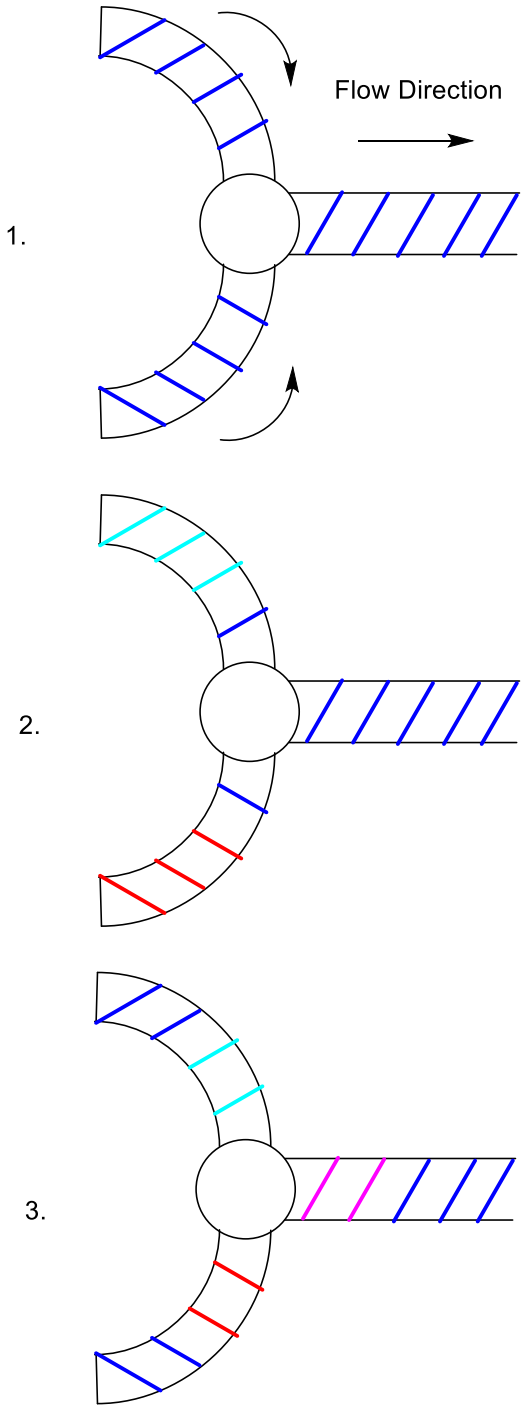
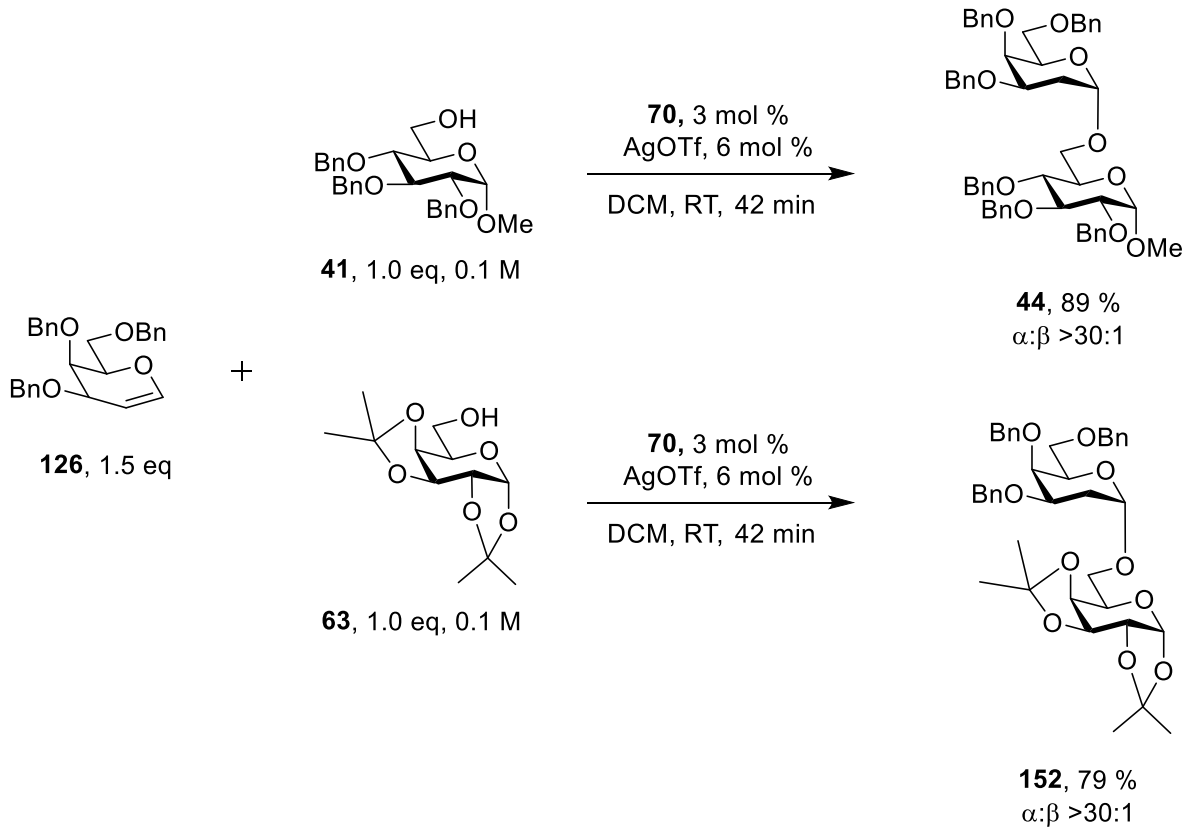


***Figure 23**. Representation of the new method for flow reactions. Two introductory tubing pieces deliver solvent to a T-mixer (large circle), leading to reactor tubing. In this representation, the dark blue lines indicate anhydrous solvent, the red and light blue lines indicate the two reactant solutions and the pink lines represent reacting solution resulting from the mixture of the red and light blue reactant solutions.*

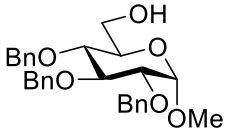
Using this method (**Figure 23**), disaccharide **151** was synthesised in 89 % yield in a 15 min residence time. At this point, it was decided to explore the scope of suitable glycosyl acceptors for the flow reaction, using the acceptors that had been reported in the batch publication. Primary alcohol acceptors **41** and **63** had been reacted with model glycosyl donor **126** to give 2-deoxy disaccharide products **44** and **152** in good yield as shown in **Scheme 54**.



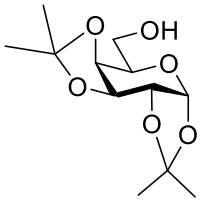
Scheme 54. *Previously reported batch reaction conditions for the gold catalysed glycosylation reaction between donor **126** and acceptors **41** and **63**.*

Following on from the excellent yield of disaccharide **151** of 89 % in a flow reaction and given the batch success of acceptors **41** and **63**, good results were expected when these acceptors were tested in flow with donor **126**. Unfortunately, this did not occur. A summary of results can be found in **Table 8**.

Table 8. *Results from attempted flow glycosylations using acceptors **41** and **63**.*



41

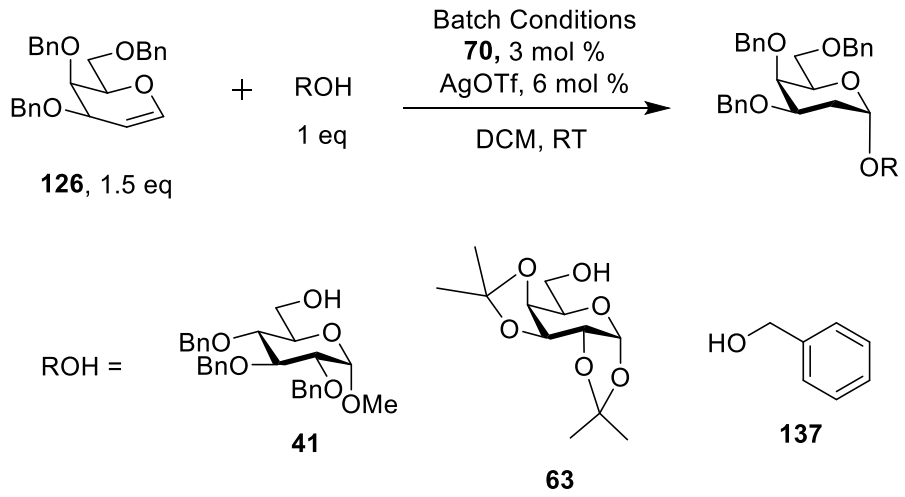


63

Entry	Acceptor	Flow Residence Time (min)	NMR Yield of Product (%)
1	**41**	15	0
2	**41**	15	11
3	**41**	30	20
4	**41**	45	30
5	**63**	15	18

Generally, very poor results were obtained, even when the residence time was increased to match that of the batch reaction as in entry 4. Repeat reactions (entry 2 compared to entry 1) did not give the same result. To determine the cause of the reaction failure, known batch reactions were repeated for acceptors **41**, **63** and for benzyl alcohol **137** with conditions matching those in **Scheme 54**. The results of these batch reaction are summarised in **Table 9**.

Table 9. *Results from batch reactions previously demonstrated to give high yields of product.*

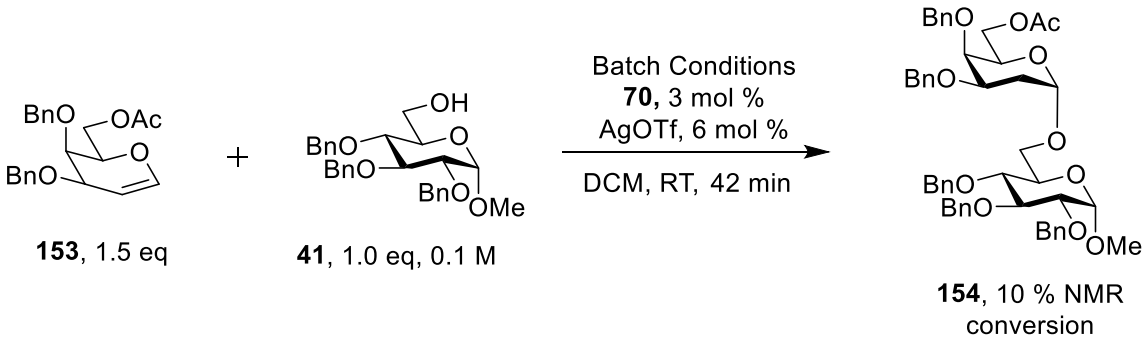


Entry	Acceptor	Reaction Time (min)	NMR Yield of Product (%)
1	**41**	45	5
2	**63**	45	85
3	**63**	50	10
4	**137**	45	0

Repetition of batch reactions reported to work well gave troubling and inconsistent results. Acceptor **41** bearing benzyl protecting groups and benzyl alcohol **137** both gave very low yields of desired product, whilst acceptor **63** bearing isopropylidene protecting groups did give the expected high yield of product (entry 2), but when the reaction was repeated (entry 3), a very low NMR yield of 10 % was obtained, without any clear explanation. The most obvious conclusion that adequately accounts for these results is that in most cases, some very small amount of impurity was poisoning the catalyst and preventing glycosylation from taking place. However, determining what this impurity might be proved exceptionally difficult, since the donor and acceptors all appeared to be of very high purity by ^{1}H NMR spectroscopy. The fact that benzoyl protected acceptor **74** works notably well, whilst no other acceptor does, was also very difficult to explain. Perhaps the donor **126** contained an impurity that was poisoning gold catalyst **70** in most cases, but benzoyl protected acceptor **74** (or another impurity contained within the sample) prevented this from happening somehow.

Alternatively, an impurity was contained in most acceptors, but not benzoyl protected acceptor **74**.

To investigate further, the role of tri-*O*-benzyl-D-galactal donor **126** was probed. Thus far, a quantity of **126** I had previously synthesised had been used.[154] This had been used successfully in the thiourea mediated organocatalytic glycosylation protocol described in section **4.2.** without any issue.[149] This ruled out that any significant amount of amine impurity was present, since it is known that amines will poison this thiourea organocatalyst and prevent glycosylation from occurring.[154] A new quantity of donor **126** was purchased from Santa Cruz Biotechnology and used in a batch reaction with acceptor **41**. ^{1}H NMR spectroscopy revealed that after 45 min, 10 % conversion to product had occurred, whilst after 2 h 20 min, 70 % conversion had occurred. This would seem to indicate that the impurity preventing glycosylation is present in acceptor **41** rather than the donor, since even with the commercial quantity of donor **126**, the reaction is much slower than had been previously reported. A reaction with a different donor, **153**, was also performed, as shown in **Scheme 55**. Previously in batch, disaccharide **154** had been obtained in 94 % yield over 42 min.[99] However, when the reaction was repeated, only 10 % conversion to product was seen by analysis of the crude ^{1}H NMR spectrum after 45 min. This further suggests that the impurity poisoning the catalyst came with the acceptor.



Scheme 55. *Batch reaction conditions for the gold catalysed glycosylation of donor* ***153****.*

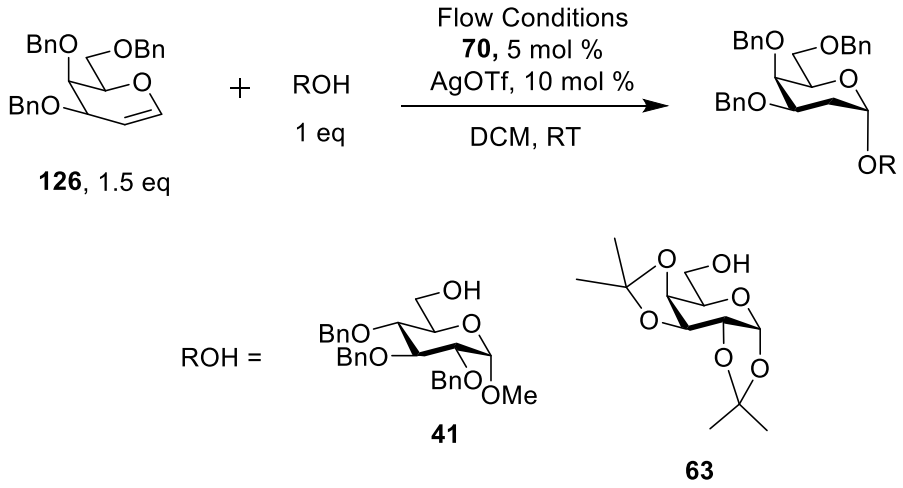
At this point, the colleague who had originally performed the reactions attempted to replicate results that he had previously obtained but came up with the same lack of success. To overcome this obstacle, new bottles of gold catalyst **70**, silver triflate and donor **126** were purchased from Sigma-Aldrich. With these new reagents to hand, the batch reaction between

donor **126** and acceptor **41** was repeated. This time, 63 % of acceptor had converted to product disaccharide **44** after 45 min. Repeating the reaction with 5 mol % of catalyst **70** and 10 mol % of silver triflate, as opposed to 3 mol % and 6 mol % as previously, gave 81 % conversion to product over 45 min, coming very close to the published results (89 % over 42 min). The yields obtained showed a marked improvement over those using the old reagents but were still marginally lower than those previously reported. These results seemed to suggest that the reason for lack of reactivity may lie with purity of gold catalyst **70**, and/or silver triflate used. The question then became, how is it that benzoyl protected acceptor **74** underwent glycosylation so smoothly with the old catalyst bottles? The answer is far from clear.

Nonetheless, with a conversion to disaccharide product **44** of 81 % seen, the conditions used in the most successful batch reaction were taken forward for use in the flow regime. In order not to change any variables that might influence the course of the reaction, DCM was used as the solvent for flow as in batch, but sonication was used to help dissolve the silver triflate. For these reactions, both the coil reactor and the newly purchased microchip reactor were used. The results of these experiments are shown in **Table 10**.

Entries 1 and 2 show that on moving from batch to flow conditions, a significant drop in conversion to product of around 20 % is seen when acceptor **41** is used. This decrease in conversion is maintained irrespective of whether the reaction is in the coil or microchip reactor (entries 3 and 4). Somewhat more positively, when acceptor **63** is used as in entry 5, 79 % conversion to product is seen with 45 min residence time. However, following these results, the decision was taken that this system is too sensitive and unreliable to be easily translated from batch to flow conditions and thus work on this project was halted.

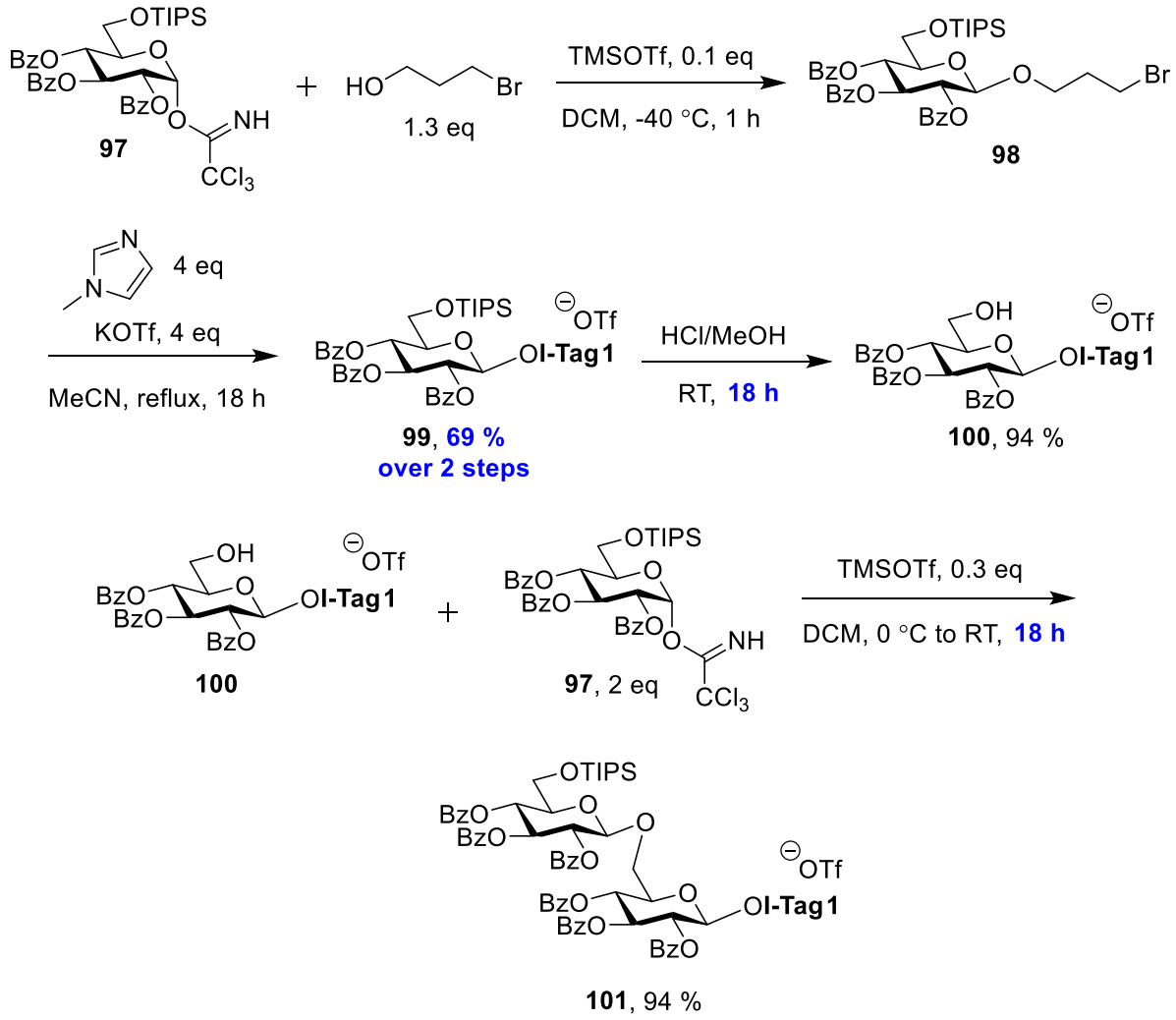
Table 10. *Results from flow reactions after having found optimised batch reaction conditions for glycosylations using gold catalyst* ***70****.*



Entry	Acceptor	Reactor Type	Residence Time (min)	NMR Yield of Product (%)
1	**41**	Coil	45	61
2	**41**	Coil	60	58
3	**41**	Microchip	30	33
4	**41**	Microchip	45	57
5	**63**	Microchip	45	79

4.6. I-Tag Supported Glycosylation in Continuous Flow

In this project, the aim was to construct oligosaccharides in flow using the iterative glycosylation-deprotection-trituration sequence with sugars bearing I-Tags. In pursuing this goal, previous publications by Galan and co-workers described in the introduction section **2.7.** served as a guide for the translation of batch glycosylation reactions to the flow regime.[144, 146] Consider once more the I-Tag supported batch experiments originally conducted by Galan and co-workers, first shown in the introduction section but reproduced here in **Scheme 56**.



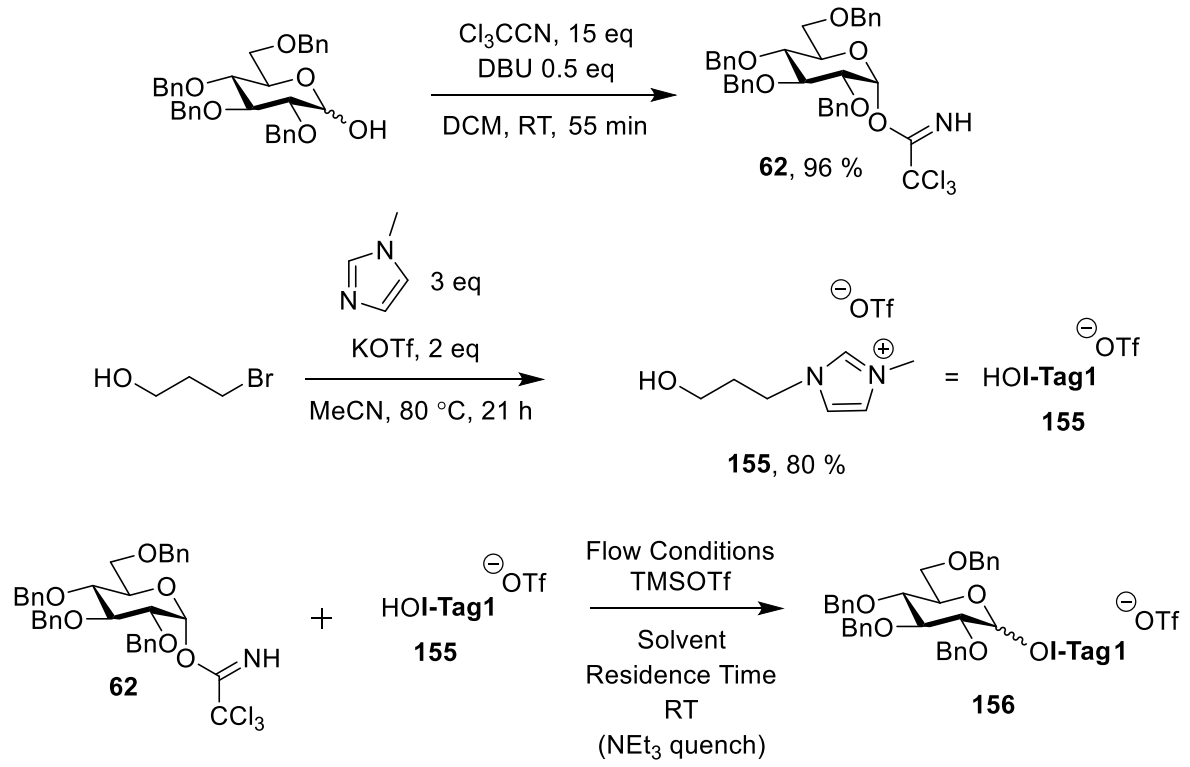
Scheme 56. *Batch ICROS reactions previously reported by Galan and co-workers. The limitations of this synthesis that might be overcome through continuous flow methods are highlighted in blue.*

This synthesis is rather elegant, but not without limitations. Transformation of glycosyl donor **97** to I-Tagged sugar **99** is completed over two steps in 69 % overall yield. Whilst this yield is reasonably high, it was hoped that this part of the synthesis could be streamlined by glycosylating donor **97** with an alcohol acceptor already bearing I-Tag functionality, so that I-Tagged sugar **99** may be accessed from donor **97** in a single step and ideally in a yield higher than 69 %. Another problem for this synthesis is that the TIPS deprotection of **99** to form glycosyl acceptor **100** and the subsequent glycosylation of **100** are both overnight reactions. Whilst the yields are very high (>90 %) for these reactions, the long reaction times limit the expediency of oligosaccharide assembly using an I-Tag strategy. Furthermore, long reaction times of 18 hours are totally unfeasible for the microflow regime, which is much better suited

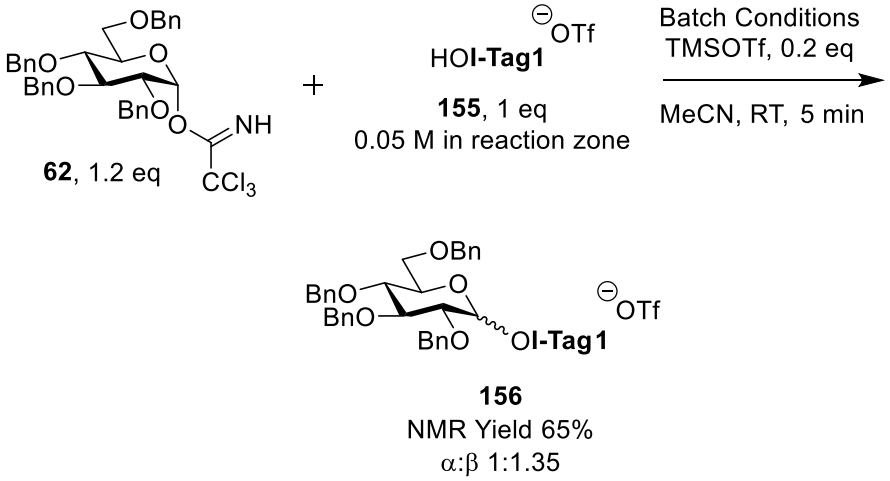
to fast reactions. Therefore, if these reactions were to be successfully translated from batch conditions to flow, reaction time would have to be dramatically reduced. The properties of flow reactors may permit faster reaction rates than those achievable in batch and so, with suitable reaction optimisation, rapid reaction times might be attainable. In this respect, the continuous flow glycosylation study reported by Lay and co-workers that was described in introduction section **2.6.** proved that very fast 1 minute glycosylations could be performed in the flow regime in a straightforward manner. This study was an encouraging indication that translation of I-Tagged batch reactions to flow, with a large decrease in required reaction time, was possible.

4.6.1. Initial Results and Optimisation of Model Reaction

The model reaction for screening flow glycosylation reactions and optimising variables is shown in **Scheme 57**. Trichloroacetimidate donor **62** can be synthesised as an anomerically pure α glycoside in one step from the commercially available free hemiacetal 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose as shown. Furthermore, being an armed donor, high reactivity would be expected and thus **62** was chosen as the model donor. Glycosyl acceptor **155** was synthesised on a multi-gram scale from 3-bromopropan-1-ol and 1-methylimidazole and used as the glycosyl acceptor. Reactions were performed in anhydrous acetonitrile since the polar I-Tagged alcohol **155** was insoluble in DCM. The glycosylation reaction shown in **Scheme 57** was firstly tested under batch conditions by adding TMSOTf to a solution of donor **62** and acceptor **155** as shown in **Scheme 58**. The NCHN and NCH_3 peaks present in both the acceptor and product provide a useful "handle" by which to determine reaction progress by ^{1}H NMR spectroscopy. Encouragingly, glycoside **156** was produced in 65 % conversion from acceptor **155**, with an α:β ratio of 1:1.35.



Scheme 57. *Model reaction chosen to explore the glycosylation of glycosyl donor* ***62*** *with alcoholic I-Tag* ***155*** *to produce I-Tagged glycoside product* ***156****.*



Scheme 58. *Initial batch reaction performed using donor* ***62*** *and acceptor* ***155****.*

With this positive batch result to hand, flow reactions and optimisation could begin following the conditions in **Scheme 57**.

4.6.1.1. Optimisation of Model Reaction in a Flow Microreactor

A beauty of flow reactions is that many different residence times can be tested rapidly by simply altering the rate at which the syringe pumps depress the plungers of the syringes containing the reaction solution. Thus, several residence times were tested for each set of reaction parameters. One solution containing the donor and acceptor and another containing TMSOTf were prepared and these solutions were flowed directly through the flow microreactor chip, as shown in **Figure 24**.

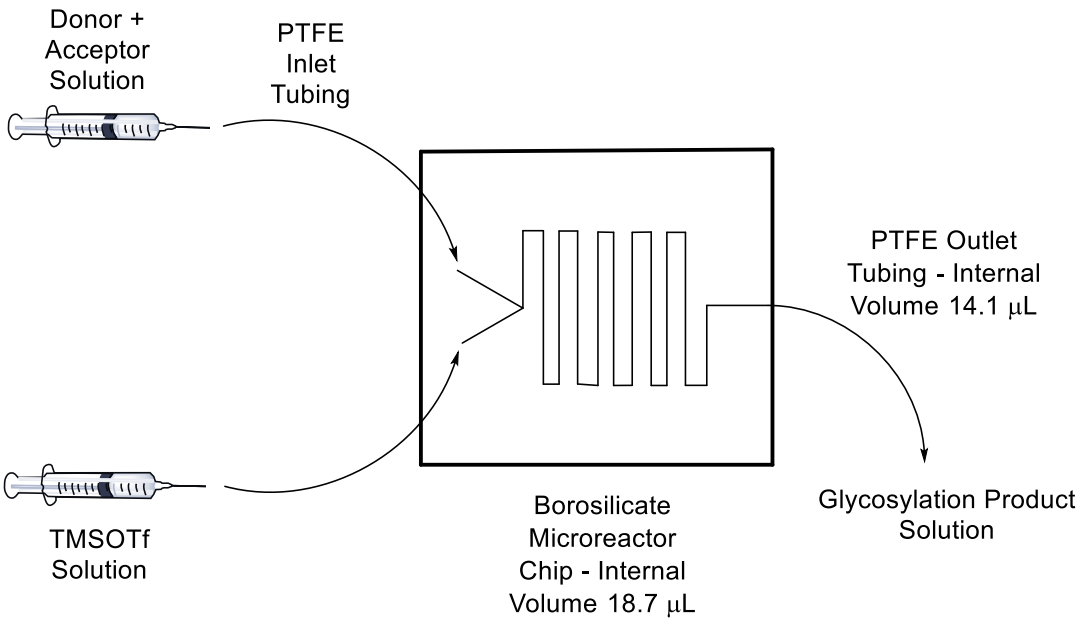
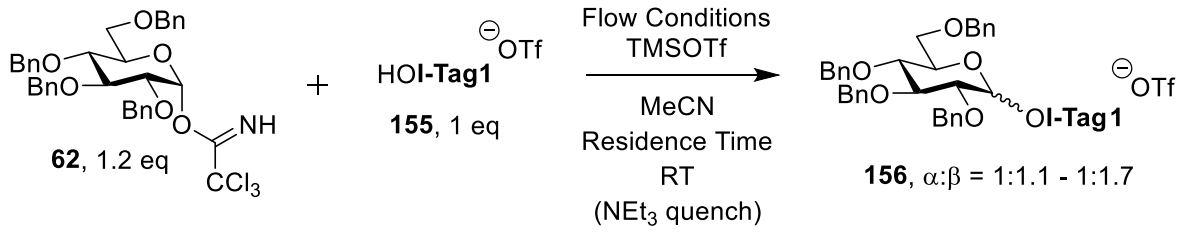


Figure 24. *The experimental setup for I-Tag supported flow glycosylations in a microreactor.*

At this stage of the project, the reaction solution leaving the outlet tubing of the flow reactor was fed directly into a solution of triethylamine in DCM to quench the TMSOTf, stopping the reaction and thus providing a precise residence time. It should be noted that the reaction will continue to occur in the outlet tubing after leaving the microreactor chip. The total volume of the reactor zone will therefore be the 18.7 μL internal volume of the chip, in addition to the 14.1 μL internal volume of a 20 cm length of PTFE outlet tubing, for a total reactor volume of 32.8 μL. This is important as the internal volume of the reactor must be known to calculate the necessary flow rate to achieve a particular residence time. In every reaction the α:β ratio of product **156** was in the range of 1:1.1 to 1:1.7. **Table 11** shows the

results of initial optimisation experiments for the model reaction, in which the influence of residence time in particular was explored.

Table 11. *Initial reactions and optimisation of residence time using the model system.*

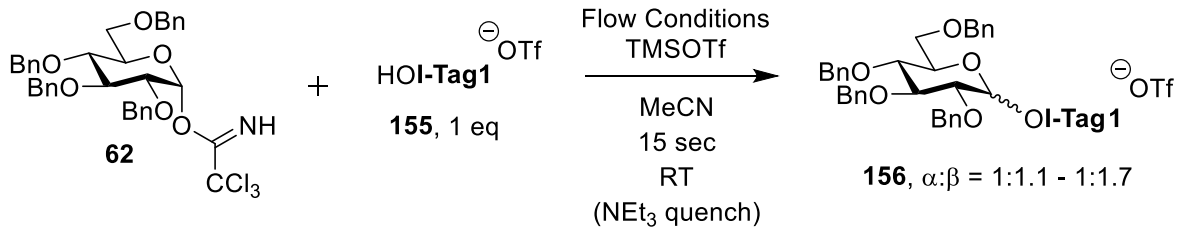


Entry	Concentration of Acceptor 155 (M)	TMSOTf (eq)	Residence Time (min)	NMR Conversion of 155 to 156 (%)
1a	0.05	0.2	5	61
1b	0.05	0.2	3	62
1c	0.05	0.2	1	62
1d	0.05	0.2	0.5	63
2a	0.05	**0.1**	5	36
2b	0.05	**0.1**	3	34
2c	0.05	**0.1**	1	48
2d	0.05	**0.1**	0.5	50
3a	**0.10**	**0.2**	5	32
3b	**0.10**	**0.2**	3	44
3c	**0.10**	**0.2**	1	66
3d	**0.10**	**0.2**	0.5	68
3e	**0.10**	**0.2**	0.25	66
4a	0.10	**0.15**	3	66
4b	0.10	**0.15**	1	69
4c	0.10	**0.15**	0.5	65
4d	0.10	**0.15**	0.25	64
4e	0.10	**0.15**	0.10	63

In entry 1, four different residence times were tested, ranging from 5 minutes down to 30 seconds. Intriguingly, the variation in residence time made virtually no difference to the conversion of acceptor to product. α:β ratios remain essentially constant across residence times. In entry 2 flow reactions use just 0.1 eq of TMSOTf rather than 0.2 eq. A reduction in

conversion to product is seen, though interestingly this decrease in yield is more severe at longer residence times, though the reason for this is not entirely clear. Because of the reduced yield in entry 2, the amount of TMSOTf was increased to 0.2 eq once again. Furthermore, the concentration of all reagents was doubled, making the acceptor 0.10 M during the reaction. Moreover, since the faster residence times seemed to give very similar or better conversions to product than the longer residence times, an additional short residence time of 15 seconds was also performed. Entry 3 shows that as in entry 2, the highest conversions are seen in residence times of 1 minute or less, whilst longer residence times showed poor conversions. Finally, in entry 4, the concentration was maintained at 0.10 M in acceptor **155**, whilst the stoichiometry of TMSOTf was adjusted to 0.15 eq. In these reactions, conversion to product was in the 60-70 % range across all residence times tested, even at a residence time of just 6 seconds. Although, at this residence time, manually operating the flow reactor and changing collection flasks becomes difficult to do quickly given the very high flow rate. The results from entry 4 in comparison with entry 2 suggests that the concentration of reacting solution has a significant influence on product formation. Overall, these data clearly show that performing reactions at longer residence times has no benefit for the reaction, so a rapid residence time of 15 seconds was tested exclusively in future reactions. Further optimisation experiments are summarised in **Table 12**.

Table 12. *Results of experiments to optimise further reaction parameters.*



Entry	Concentration of Acceptor 155 (M)	Donor 62 (eq)	TMSOTf (eq)	NMR Conversion of 155 to 156 (%)
1	0.10	**1.5**	0.15	67
2	0.10	**2.0**	0.15	79
3	0.10	2.0	**0.3**	85 (84)[a]
4	**0.05**	2.0	0.3	72
5	**0.10**	2.0	**0.5**	80
6	0.10	**3.0**	**0.6**	81

[a]Isolated yield.

In entry 1, the amount of donor used is increased to 1.5 eq from 1.2 eq, whilst 0.15 eq of TMSOTf is used as previously. At a residence time of 15 seconds, 67 % conversion to product is seen. This is essentially the same as the 64 % conversion seen in entry 4d from **Table 11**. By further increasing the amount of donor used to 2.0 eq as in entry 2, an increase in conversion to product from 67 % to 79 % is observed. Entry 3 shows that using 0.3 eq TMSOTf, an excellent conversion to product of 85 % is observed, especially given the extremely short reaction time. This is even more remarkable considering that for the batch reactions performed earlier in the group, the glycosylation of an I-Tagged glycosyl acceptor was an overnight reaction. Entry 4 confirms that reducing concentration is detrimental to yield, with 72 % conversion compared to 85 % in entry 3. In entry 5, a higher loading of TMSOTf of 0.5 eq was used. This gave a slightly reduced conversion to product of 80 % compared to entry 3. Finally, entry 6 shows that further increasing the amount of donor to 3 eq as well as TMSOTf to 0.6 eq does not give higher conversion to product than the conditions from entry 3. The conditions leading to highest conversion to product were those found in entry 3, in which the acceptor was 0.10 M in the reactor zone, 2 eq of donor and 0.3 eq TMSOTf were used and the residence time was set to 15 seconds. The product **156** was obtained in high purity from the reaction shown in entry 3 with purification achieved through

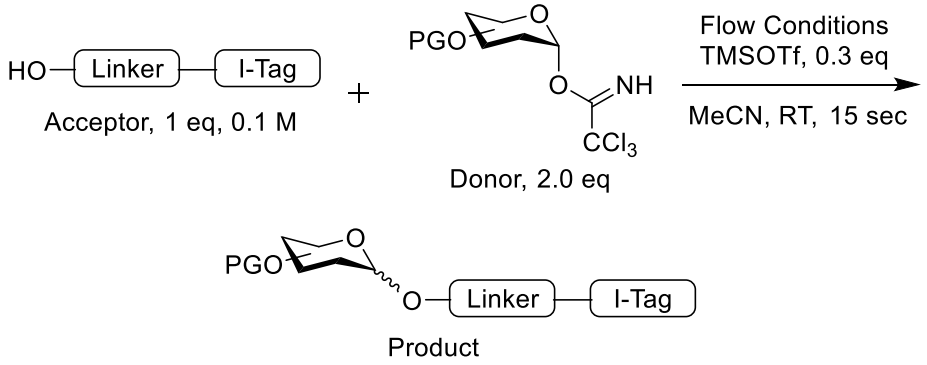
simple washes using firstly water, then hexane:Et_2O 1:1 to give the product in 84 % isolated yield and α:β ratio of 1:1.6. The optimised reaction conditions were used as the basis for subsequent exploration of substrate scope for this flow reaction protocol.

4.6.2. **Exploration of Substrate Scope**

Several glycosyl donors and acceptors were now subjected to the optimised reaction conditions discovered during the optimisation of the model reaction, as shown in **Scheme 59**. The results of these experiments are summarised in **Table 13**. Isolated yields reported are following purification by solvent washing, with no requirement for column chromatography.

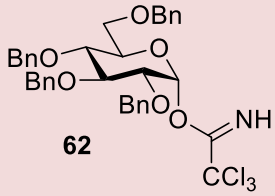
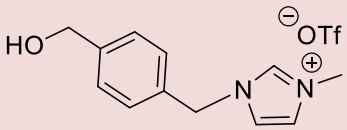
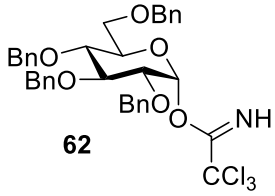
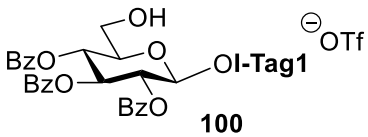
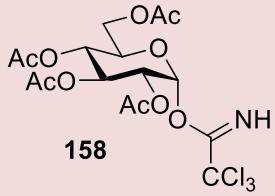
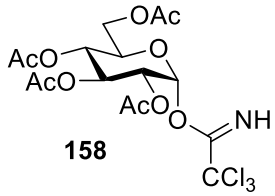
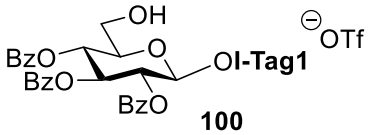
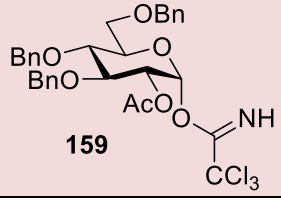
Peracetylated donor **158** was obtained from a colleague and used in these experiments directly, whilst donor **159** was synthesised from tri-*O*-benzyl-D-glucal **36** in 26 % yield over four steps, featuring a selective dihydroxylation reaction across the enol ether double bond. In entry 1, model donor **62** is reacted with benzyl linked I-Tagged alcohol **157** to furnish the product saccharide as an anomeric mixture in 90 % isolated yield. Entry 2 shows that the reaction between donor **62** and saccharide derived I-Tagged alcohol **100** also proceeds smoothly, giving the desired disaccharide product in 86 % isolated yield. However, no product could be isolated when peracetylated glycosyl donor **158** was used as in entries 3 and 4. ^{1}H NMR spectroscopy revealed a complex mixture, whilst TLC showed no spots corresponding to product. Finally in entry 5, the reaction between donor **159** and acceptor **155** was performed, with the resulting product being isolated in 73 % yield as the pure β anomer, owing to the neighbouring group participation of the C-2 acetate group in donor **159** during the glycosylation reaction.

The concept of armed and disarmed glycosyl donors may help to explain the results shown in **Table 13**. Armed donor **62** consistently undergoes reaction smoothly, whilst disarmed donor **158** does not give appreciable product formation under these conditions. Donor **159** is electronically super-armed in the manner reported previously by Demchenko and co-workers by using a participating ester protecting group at C-2, and arming benzyl ether groups at C-3,4 and 5. As may be expected, this reactive donor gave the desired product in good yield.



Scheme 59. *Optimised conditions used for exploration of the substrate scope of I-Tag glycosylation in flow.*

Table 13. *Results of flow glycosylation experiments using different glycosyl donors and acceptors.*

Entry	Donor	Acceptor	Result
1[a]	 **62**	 **157** = HO**I-Tag2** **157**	90 %, α:β 1:2
2	 **62**	 **100**	86 %, α:β 1:2.3
3	 **158**	HO**I-Tag1** **155**	Complex mixture.
4	 **158**	 **100**	Complex mixture.
5	 **159**	HO**I-Tag1** **155**	73 %, β only

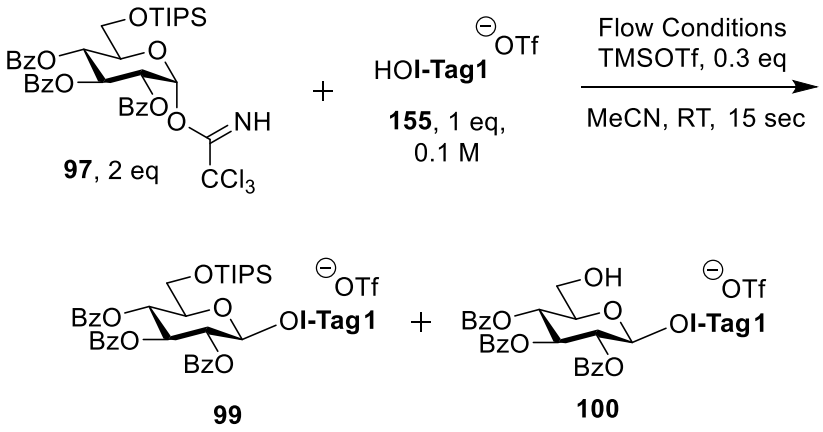
[a]Reaction performed with 3 eq donor and 0.6 eq TMSOTf.

At this point in the project, the use of mass spectrometry as a tool for the analysis of crude reaction mixtures became important, especially since the reactions that did not

proceed smoothly produced a complex mixture that was difficult to analyse accurately by NMR spectroscopy. However, it is not possible to accurately determine a percentage yield value from mass spectrometry, since the signal intensity observed is affected by several factors, not just quantity of compound. Furthermore, at this point, it became apparent that triethylamine was a potentially poor choice as a quenching agent for this reaction. This is because the triethylammonium salts that can form upon quenching may be difficult to separate from the I-Tag, which is itself an ammonium salt. Instead, it was reasoned that contact with water in the atmosphere and in the reagent grade solvent in the collection flask would be sufficient to stop further reaction from occurring. Evidence acquired by monitoring the reaction mixture exiting the flow reactor by ^{1}H NMR spectroscopy confirmed this hypothesis, since no change in ratio of starting material and product was seen over time, even with no triethylamine or other chemical quenching compound added to the receiving flask. This indicated that no further reaction was occurring after the solution exited the outlet tubing of the flow reactor, and hence the calculated residence time was accurate.

4.6.3. **Reactions Using Triisopropylsilyl Ether Protected Donors**

Next, the reaction between orthogonally protected donor **97** and I-Tagged alcohol **155** was tested. The use of donor **97** is important, as complete or at least very high anomeric selectivity, achieved in this case through neighbouring group participation, is imperative for oligosaccharide assembly. Moreover, the presence of an orthogonal protecting group at C-6 is essential for the glycosylation-deprotection-trituration sequence to prepare β-1,6-glucans. The results of the experiment are shown in **Scheme 60**.



Scheme 60. *Outcome of the reaction between donor **97** and acceptor **155** using the optimised reaction conditions.*

Since donor **97** was used extensively in previous publications, the expectation was that this reaction would work. However, the results from the reaction were disappointing. TLC indicated that two species were present that had a polarity in the range that would be expected for I-Tag product **99**, though both appeared to be formed in very low yield, with the majority of carbohydrate material visible by TLC being of a polarity that would be expected for a neutral protected carbohydrate molecule. NMR analysis of the crude reaction product mixture revealed predominantly unreacted acceptor **155** was present. Trituration of the crude material using water, then hexane/diethyl ether was performed. NMR analysis of the residue revealed two species that appeared largely similar by NMR, that were not anomers. A LC-MS experiment elucidated the identity of the two species. One species was the desired product **99**, the other was TIPS-deprotected product **100**. It appeared that under the reaction conditions, the TIPS group could be deprotected during the reaction. However, there was no evidence by mass spectrometry that 6-hydroxyl product **100** had reacted with another molecule of **97** to form a disaccharide. Since the deprotection of the TIPS group was the next step in the synthetic pathway, if the TIPS deprotection was not competitive with the desired glycosylation reaction, it was reasoned that this deprotection may not be an issue. However, at this stage, the products **99** and **100** could not be fully purified. In order to ameliorate the reaction using orthogonally protected donor **97**, further reaction conditions were screened moving forward.

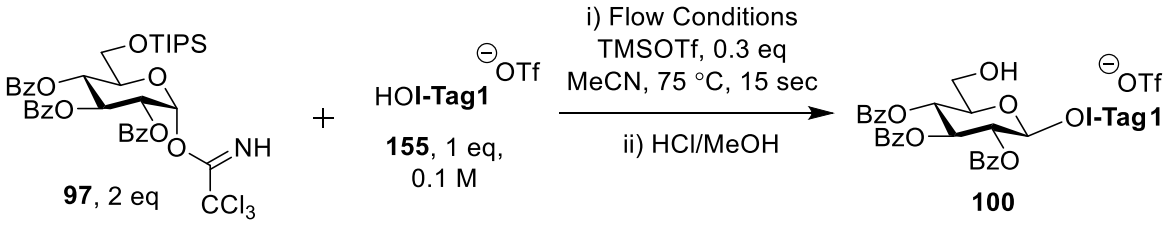
The use of TfOH as the activator in the next reaction in place of TMSOTf gave virtually identical results to using TMSOTf. The crude product was analysed by LC-MS, which indicated that of the glycosylation product that formed, approximately 58 % still bore the TIPS group, whilst 42 % had lost the TIPS to furnish the free alcohol. Following trituration with hexane/diethyl ether, the residue was dissolved in a solution of HCl in methanol to fully deprotect the product. Purifying the resultant alcohol **100** by trituration yielded pure product as the β anomer, but in very low yield (<10%). Experiments continued to try and improve the yield of the reaction.

In future experiments between donor **97** and acceptor **155**, the outlet tubing from the flow reactor was fed directly into a methanolic HCl solution to immediately complete TIPS deprotection whilst simultaneously halting glycosylation. Further experiments indicated that the product can degrade if exposed to aqueous sodium hydroxide, hence the acidic methanol should not be neutralised, but simply transferred to an aqueous phase through dilution with water and DCM, retaining the product in the DCM phase. In the next experiment, after purification by trituration, I-Tagged alcohol **100** was obtained in less than 15 % yield and could not be entirely purified, with small amounts of impurity remaining in the sample. It appeared that there was no way to increase the yield to useful levels. Another issue was that during some of the reactions between donor **97** and acceptor **155**, solid particles began to form in the microreactor chip and/or the outlet tubing, preventing solution flow. This led to irreversible blockage of the reactor. Considering the persistently poor yields amongst other problems, the reaction conditions were re-evaluated to ensure they were optimal.

Thus far, the only variable that had not been changed that was likely to cause a meaningful increase in product yield was temperature. It was reasoned that in the reaction between the disarmed, less reactive donor **97** and acceptor **155**, an increased reaction temperature might help the reaction to progress further, whilst stopping the insoluble particles from precipitating out of solution. To elevate the reaction temperature, the reactor was submerged in an oil bath at 75 ˚C. Since only the reactor, not the outlet tubing, was at 75 ˚C, the residence time was adjusted to use the 18.7 μL reactor chip as the internal volume, whilst the outlet tubing reaction at room temperature was considered as “background reaction”. It is also probable that the glycosylation would be complete by the time the reacting solution entered the outlet tubing at RT after spending 15 seconds in the reactor chip

at 75 ˚C. In some cases, the residence time was reduced from 15 seconds to 10 or 6 seconds. This was done to keep flow rate high and prevent the solid particles from precipitating in the reactor, though previous evidence acquired during the optimisation of the model reaction suggests that a small change in residence time would have a very minor effect on the reaction. The 75 ˚C experiments performed are summarised in **Table 14**. In each of the five entries, the same reaction was performed, except with small alterations in residence time as described.

Table 14. *Flow glycosylation experiments performed at 75 ˚C using donor **97** and acceptor **155** with immediate acidic silyl ether deprotection to give product **100**.*

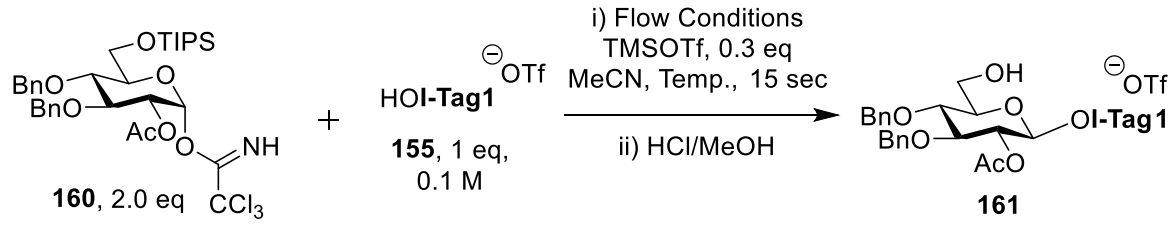


Entry	NMR Yield of Product 100 (%)
1	54
2	21
3	38
4	14
5	12

Perhaps the most striking feature of the results contained in **Table 14** is the huge discrepancies in yield encountered for the same reaction. Additionally, whilst the high temperature prevented solid particles from forming in the reactor, they did sometimes form in the much cooler outlet tubing, blocking it. Overall, very poor conversions were achieved in this reaction, despite this donor working well in batch reactions in previous work.

Alongside experiments using donor **97**, experiments were also performed using super-armed donor **160** (**Scheme 61**). The reasoning behind this was that some success had been

achieved using super-armed donor **159** and therefore another super-armed donor might work well. When this donor was tested using the optimised reaction conditions at room temperature, the yield of product **161** was only 14 % (**Table 15**). Further reactions altered the reaction temperature to both higher and lower reaction temperatures. The logic behind these changes was that a higher temperature might encourage reaction if the reactivity of the substrates was low. Conversely, if the donor was highly reactive, it could be reacting intramolecularly or rearranging before glycosylation could happen, in which case lowering the temperature might stabilise the donor in order that glycosylation is able to take place. Indeed, this appeared to be the case, since the NMR yield of product **161** at 75 ˚C was 11 %, increasing to 18 % when the reaction temperature was lowered to -10 ˚C. Ideally, the temperature would have been lowered further still, but -10 ˚C was found to be the minimum accessible temperature. This was because lowering the temperature any more caused the solutes to precipitate out of solution in the flow reactor.



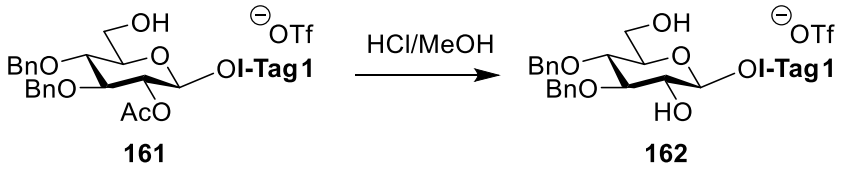
Scheme 61. *Reaction conditions used when investigating donor **160** as a suitable donor for flow glycosylations of I-Tags.*

Table 15. *Flow glycosylation experiments performed at different temperatures using super-armed donor **160** following the conditions shown in **Scheme 61**.*

Entry	Temperature / ˚C	NMR Yield of Product 161 (%)
1	RT	14
2	75	11
3	-10	18

One observation from experiments using donor **160** was seen that led to an improved silyl ether deprotection step. As in the case of donor **97**, after the flow glycosylation reaction was performed, a mixture of I-Tagged product still bearing the TIPS group and product **161**

that had lost the TIPS group were present in the crude residue from each reaction. This mixture was subjected to a methanolic solution of HCl to effect complete TIPS deprotection, a reaction generally taking at least 3 hours to complete. However, this led to an undesired side product observed by LC-MS. The mass of the product and its polarity relative to **161** suggested the loss of an acetyl group, and thus the side reaction that leads to this product is postulated in **Scheme 62**. Acid catalysed transesterification of the *O*-2 acetyl protecting group with methanol was observed, leading to an inseparable mixture of acetyl protected **161** and deprotected diol **162**. To overcome this obstacle, a solution of HCl in diethyl ether was used instead. Since ethers are much less nucleophilic than alcohols, it was hypothesised that this solvent would keep the acetate protecting group intact. This turned out to be correct and no acetate group removal was seen by mass spectrometry. Furthermore, the TIPS deprotection proceeded much faster in ether than in methanol, with reaction times reduced to only 10-30 min. It should be noted that addition of water to the HCl/ether solution degraded the saccharide product **161**, therefore, the workup first requires evaporation of the HCl/ether solution under reduced pressure prior to aqueous washes.



Scheme 62. *A solution of HCl in methanol can cause undesired deprotection of the C-2 acetate protecting group.*

Although several important discoveries had been made, the general story was a disappointing lack of high yields with any donors apart from the model donor **62** and super-armed donor **159** (**Figure 25**). It became apparent that a constant factor in the donors that hadn't worked (apart from peracetylated donor **158**) was the TIPS protecting group at C-6. An especially stark difference in reactivity was observed between super-armed donors **159** and **160**, which are differentiated solely by the C-6 protecting group. It was reasoned that the TIPS group is rather sterically bulky, obstructing the β face of the sugar ring. It also tends to be deprotected during the flow reaction, potentially inhibiting the glycosyl donor from being glycosylated.

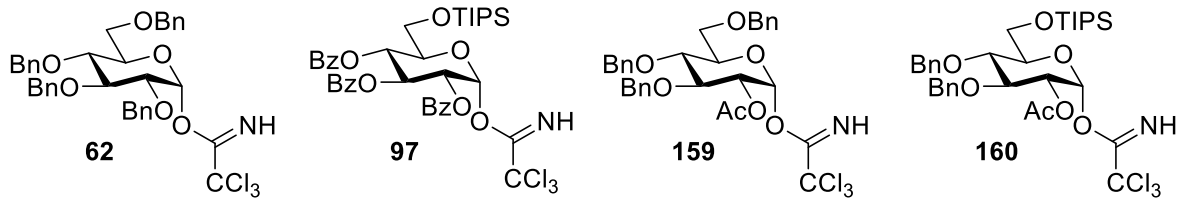
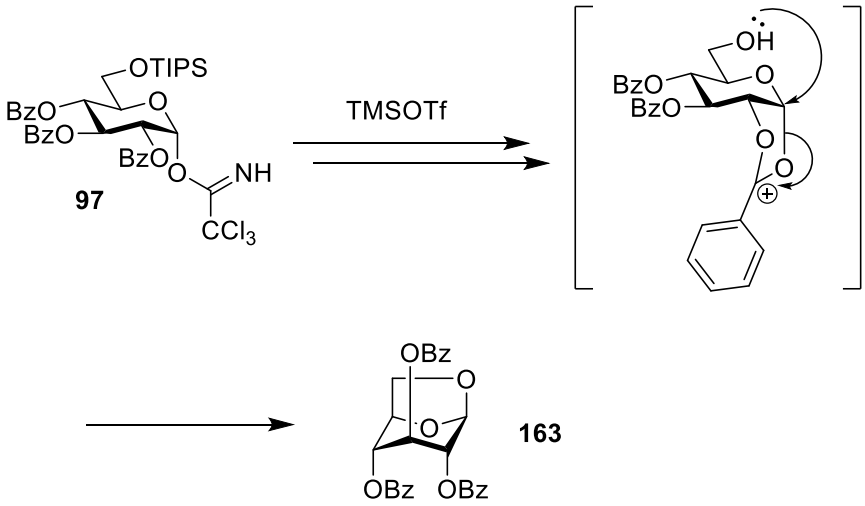


Figure 25. *Donors used in flow glycosylations.*

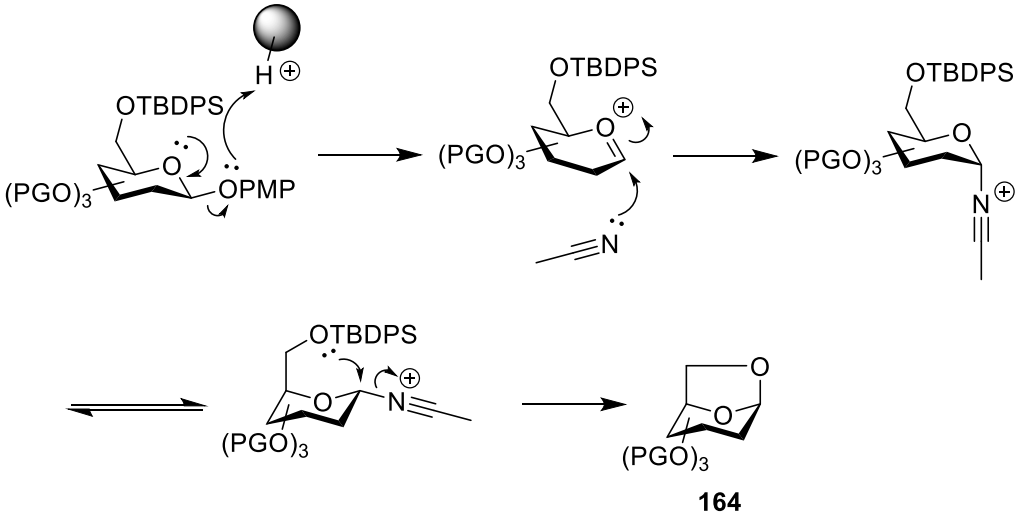
To investigate this idea further, the identity of the unknown solid compound that had tended to block the reaction tubing during flow glycosylations with benzoyl protected donor **97** was elucidated. To do this, donor **97** was dissolved in dry acetonitrile in a vial to make a solution of the same concentration as was used for the flow reactions, to which TMSOTf was added directly. The mixture was swirled for 3 minutes, then reagent grade "wet" acetonitrile was added along with air exposure to quench the reaction. The solid compound did not precipitate from solution directly but appeared when the solvent was evaporated. The crude product was purified by washing with a small amount of acetonitrile three times. The unknown compound was insoluble in water, but soluble in DCM and chloroform. NMR spectroscopy and MS analysis elucidated the identity of this compound as 1,6-anhydro sugar **163** (**Scheme 63**). A particularly diagnostic characteristic for this compound was the change in *J* coupling values in the ^{1}H NMR spectrum for H-2,3 and 4 according to the Karplus equation, as the six membered chair conformation switches from the $^{4}C_{1}$ conformer to the $^{1}C_{4}$ conformer. Under the reaction conditions, it appears that the Lewis acid TMSOTf activates the trichloroacetimidate group as expected. However, if the TIPS group has been lost to give a free alcohol at *O*-6, it appears that this alcohol group can act as a nucleophile competitively with acceptor **155**. Intramolecular formation of a glycosidic bond furnishes 1,6-anhydro sugar **163**. In this case, the lack of reactivity in the intermolecular glycosylation for donors **97** and **160**, both of which have an *O*-6 TIPS group, is well explained, since they are reacting intramolecularly before intermolecular glycosylation can occur, limiting yields of desired product to usually 10-20 %.



Scheme 63. *Suggested mechanism for the intramolecular transformation of glycosyl donor* ***97*** *to 1,6-anhydro-β-D-glucose-2,3,4-tri-O-benzoate* ***163*** *with Lewis acid TMSOTf.*

At this point, the conspicuous question that arose was: why is it that the TIPS group is totally stable to TMSOTf during batch reactions at room temperature overnight, but is not stable with the same reagents in flow? The reaction being performed in flow was clearly not essential, since 1,6-anhydro sugar **163** was synthesised in a "batch" vial. Most other reaction parameters, including temperature and solution concentration were the same or similar in the flow reactions compared to the batch reactions. Therefore, the only difference envisaged to be influential was solvent. Presumably DCM, used during previously performed batch reactions, must protect against TIPS cleavage, where acetonitrile does not. A publication by Li and co-workers describes similar reactivity in the formation of 1,6-anhydro sugars from 6-*O*-TBDPS protected substrates using perchloric acid supported on silica.[160] The authors suggest a mechanism (**Scheme 64**) by which an oxocarbenium ion is generated through acid catalysed departure of the anomeric leaving group to produce an oxocarbenium ion. The intermediate ion is stabilised by interaction with the acetonitrile solvent, allowing conformational switching from the $^4C_1$ conformer to the $^1C_4$ conformer, which can then undergo intramolecular nucleophilic substitution to give 1,6-anhydro sugar **164**. It is likely that a similar mechanism is responsible for the formation of 1,6-anhydro sugars in the I-Tag supported flow glycosylation system, suggesting that the acetonitrile solvent might be responsible for the differences in reactivity between the flow glycosylations and the batch glycosylations in DCM performed previously. Although, this explanation does not account for

the anchimeric assistance from the C-2 benzoyl group, which is also likely to stabilise the oxocarbenium ion in a similar way to acetonitrile solvent.



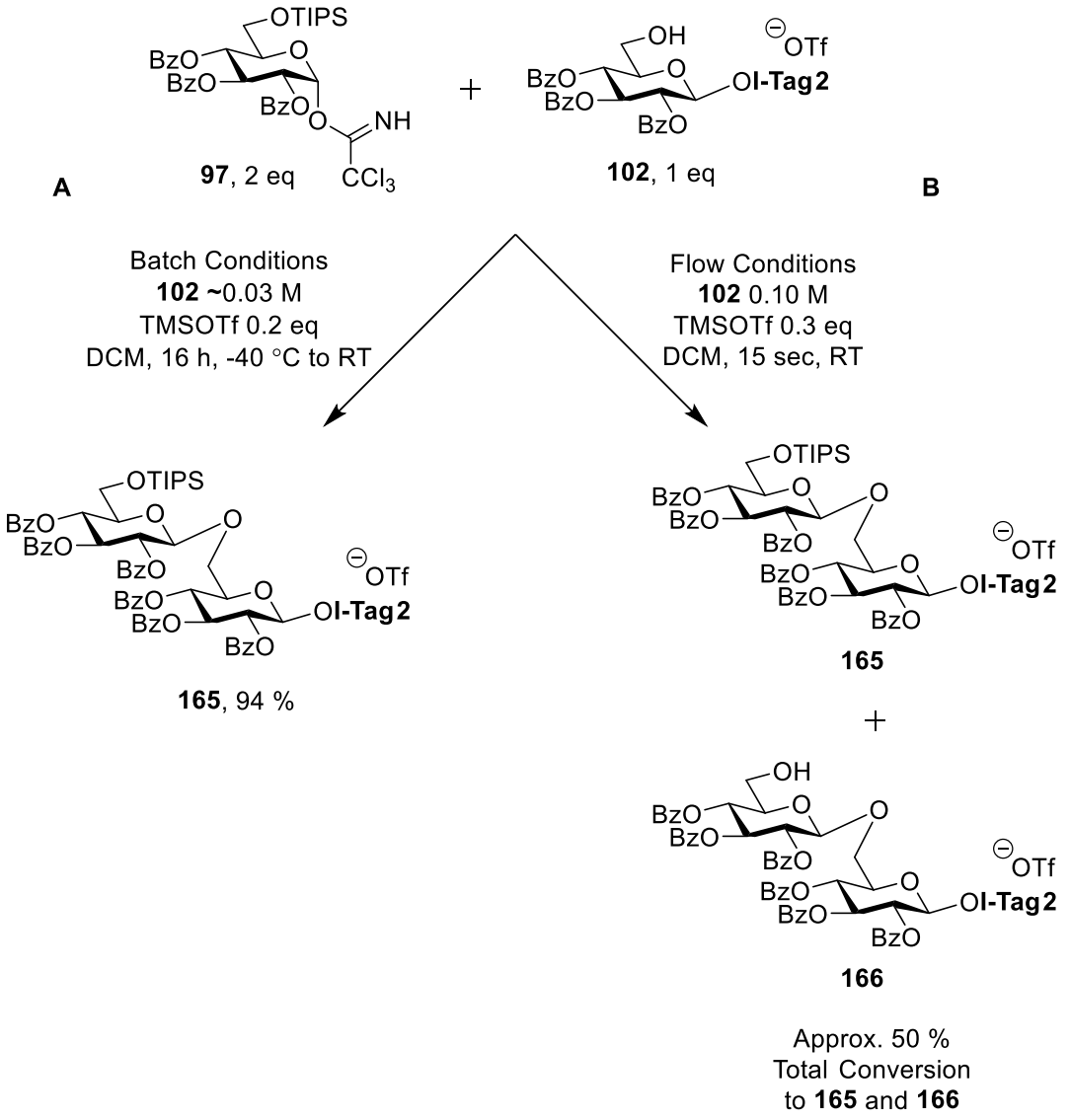
Scheme 64. *Mechanism proposed by Li and co-workers for the formation of 1,6-anhydro sugars* ***164*** *from the corresponding 6-O-silyl ether protected glycosides.*[160]

TIPS protected donor **97** was treated with TMSOTf in DCM to learn whether 1,6-anhydro sugar **163** is formed and determine whether the solvent is the key issue. It turned out that using DCM as the solvent, some product **163** is indeed formed as determined by qualitative ^{1}H NMR analysis, amongst other products, but not as completely as in acetonitrile. It appears that since DCM does not interact with the oxocarbenium ion to the same extent as acetonitrile, it may prevent TIPS deprotection and formation of **163**, however, this prevention is not complete. Presumably during a glycosylation reaction using a TIPS-bearing donor such as **97**, the formation of 1,6-anhydro product **163** and the formation of the desired glycoside are in competition. The solvent can influence which product is favoured, with acetonitrile favouring intramolecular reaction to make **163** whilst DCM favours intermolecular glycosylation.

To further investigate the influence of solvent on a glycosylation in flow, a reaction using glycosyl acceptor **102**, featuring benzyl-linked **I-Tag2**, with donor **97** was performed. Acceptor **102**, unlike the more polar acceptor **155**, is soluble in DCM and so a flow reaction using DCM as solvent could be performed. It was thought that under these conditions, high yields of glycosylation product might be obtained using donor **97**, as was the case in batch

reactions. Unfortunately, this was not the case, as shown in **Scheme 65**. Pathway **A** shows the result of a previously performed reaction in batch. Under these conditions, disaccharide **165** is formed in high yield, with no loss of the TIPS group.[146] Conversely, pathway **B** shows the result of a flow glycosylation I performed under very similar conditions, using DCM as the reaction solvent. A mixture of disaccharides **165** and TIPS-deprotected **166** were observed, with a combined conversion from acceptor **102** of approximately 50 %. Furthermore, I performed a flow experiment using disaccharide **166** as glycosyl acceptor with donor **97** to make a trisaccharide. The trisaccharide was observed once again as a mixture of product still bearing the TIPS group and its desilylated counterpart, but in just 42 % combined conversion compared to 94 % isolated yield of the TIPS-bearing trisaccharide using the same reagents in batch.

Whilst the conversion to product of 40-50 % using DCM as the solvent in the flow reactions is significantly better than the 10-20 % seen when MeCN is used, it is substantially worse than the very high batch yields of product previously reported. Presumably the low conversion in flow results from the donor reacting intramolecularly to form 1,6-anhydro sugar **163**, despite DCM being used as solvent rather than MeCN. Furthermore, partial loss of the TIPS group on the glycosyl product appears to be unavoidable in flow, regardless of solvent choice, but is not observed whatsoever in batch. These curious results highlight that the continuous flow regime seems to open and exploit new regions of chemical reactivity not observed under batch conditions. Whilst in this instance the observed reactivity was unwelcome, it is noteworthy that the flow regime not only changes the rate of reactions but can also alter the fundamental chemistry that occurs compared to batch.



Scheme 65. *Batch and flow reactions in DCM using the same glycosyl donor and acceptor.* ***A.*** *Conditions and result of previously performed batch reaction using donor* ***97*** *and saccharide derived acceptor* ***102****.*[146] ***B.*** *Flow reaction I performed using the same donor and acceptor.*

4.6.4. **Consideration of Alternative Protecting Group Strategies**

In order to move forward with the project, new glycosyl donors were required. The choice of protecting groups was paramount and had to meet several criteria. Firstly, the *O*-2 protecting group had to be able to participate anchimerically during the glycosylation reaction, or a mixture of anomers could be formed. Secondly, the *O*-6 protecting group had to be installed selectively on *O*-6. Thirdly, the *O*-6 protecting group had to be stable under the glycosylation conditions, or the undesired 1,6-anhydro sugar could form again. Fourthly, the

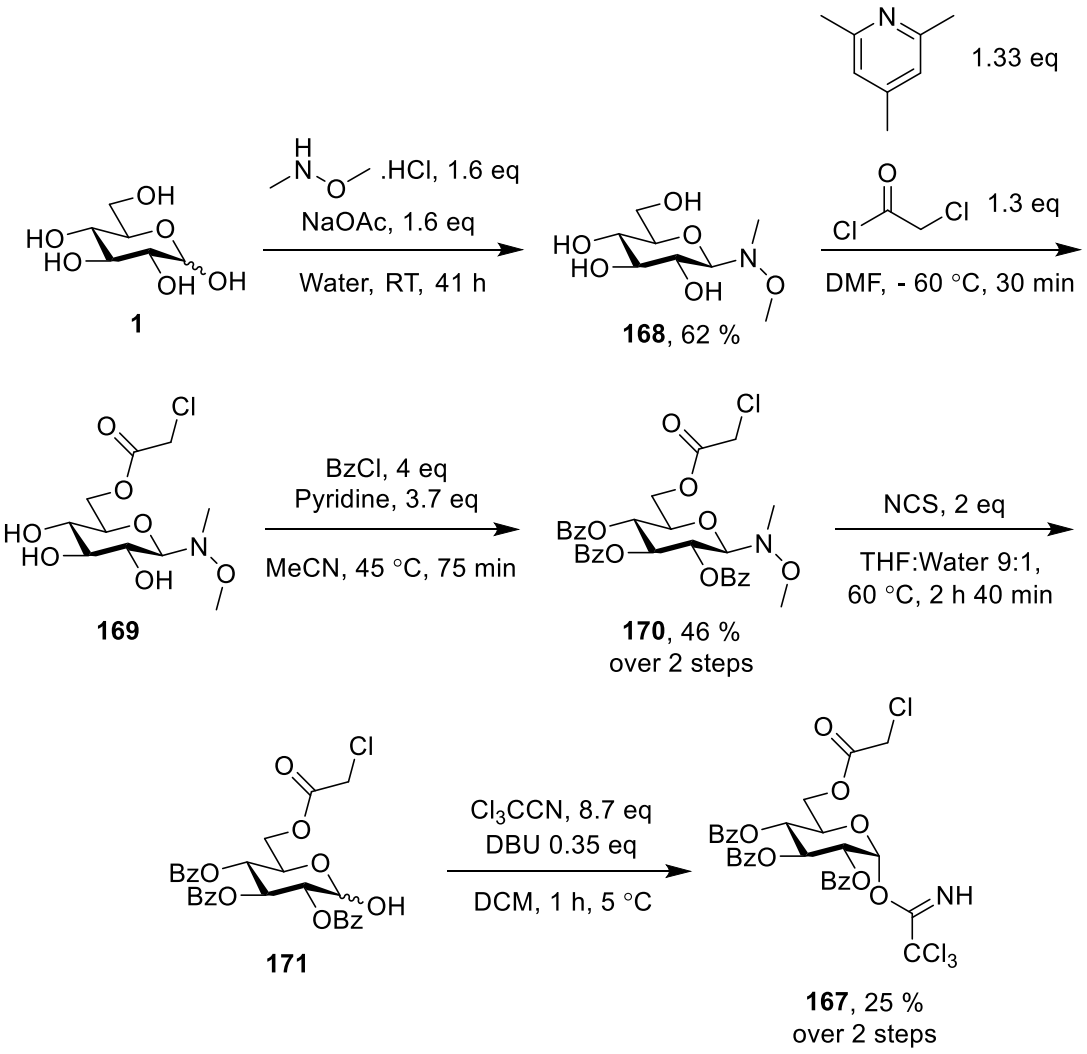
O-6 protecting group had to be stable during manipulations of other protecting groups around the molecule. Finally, the *O*-6 protecting group had to be orthogonal or semi-orthogonal to the protecting groups at *O*-2,3 and 4 so that it could be selectively removed to permit oligosaccharide assembly.

Several protecting groups initially appeared to be sensible choices, but upon closer inspection do not meet all the criteria listed. *para*-Methoxy benzyl ethers are easy to install and stable to many reaction conditions used in chemical carbohydrate synthesis, whilst removal with one electron oxidising agents such as ceric ammonium nitrate (CAN) or DDQ is generally selective and high yielding.[8] However, literature reports suggest they are not always stable with respect to TMSOTf,[161] and thus the undesired 1,6-anhydro product could result. The *tert*-butyldiphenylsilyl ether (TBDPS) protecting group can be installed selectively at *O*-6 in the presence of secondary hydroxyl groups and is more stable towards acid catalysed hydrolysis than the TIPS group. However, the publication by Li and co-workers indicates the TBDPS group could also be cleaved under the reaction conditions, likely making it unsuitable.

A good choice for the *O*-6 protecting group might be an allyl ether, since they can be selectively removed in the presence of most other protecting groups using a heterogeneous palladium catalyst. One potential problem with this group is unwanted reactivity during deprotection of anomeric protecting groups. Common anomeric protecting groups such as thioethers or *para*-methoxyphenyl ethers are usually deprotected using *N*-bromosuccinimide (NBS) and CAN respectively. Both reagents are likely to react with the C=C double bond found in the allyl ether and so an allyl ether group may be synthetically challenging to install orthogonally. Finally, the chloroacetate group was considered as an *O*-6 protecting group. It ought to be stable towards TMSOTf, is reasonably straightforward to install, and can be removed selectively in the presence of other ester protecting groups such as benzoyl or acetate groups.[162]

Acetyl protecting groups are quick and easy to install, for example on *O*-2,3,4, however, if C-6 has an unprotected free alcohol, *O*-4 acetate groups are known to frequently migrate to *O*-6. Thus benzoyl, or perhaps pivaloyl (*tert*-butyl) esters are better suited as *O*-2,3,4 protecting groups, since they are more stable with respect to migration.

When all the above considerations were taken into account, it was decided that donor **167** should be a suitable donor for construction of β-1,6-glucans in flow, and thus it was synthesised as shown in **Scheme 66**. Donor **167** can be synthesised in five steps from D-glucose **1**. Whilst yields are generally fairly low, this is partially offset by the small number of steps required to synthesise the donor from simple starting materials. Anomeric protection using the *N,O*-dimethylhydroxylamine group can be performed on glucose directly in water to give **168** in 62 % yield. The chloroacetate group can then be regioselectively introduced to the primary C-6 alcohol at low temperature to give **169**, with subsequent perbenzoylation affording fully protected **170** in 46 % yield over two steps. Removal of the anomeric protecting group with NCS followed by formation of the trichloroacetimidate group furnishes donor **167** in 25 % yield over two steps. The low yield seen here is primarily due to loss of compound whilst performing column chromatography of donor **167**. It is known that trichloroacetimidate donors can degrade when exposed to mildly acidic silica gel. Generally, it is possible to circumvent this problem by using a small volume of triethylamine in the column chromatography solvents to neutralise the silica. However, in this instance, that strategy was ineffective, since the isolated yield was low regardless of silica neutralisation. Nevertheless, with donor **167** in hand, it could be tested for its suitability in flow glycosylation reactions.

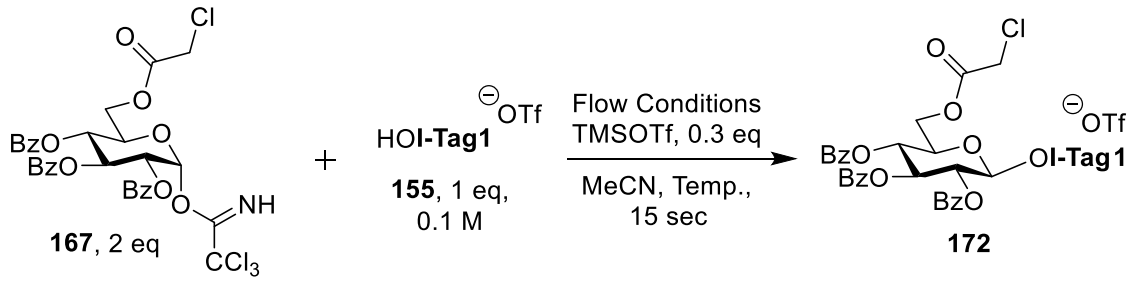


Scheme 66. *Synthetic route to make donor **167** from glucose **1**.*

4.6.5. Influence of Temperature on Glycosylations

The reaction of donor **167** with acceptor **155** to give I-Tagged glycoside **172** using the optimised flow reaction conditions at RT pleasingly showed 85 % conversion to product, present entirely as the β anomer, as shown in entry 1 of **Table 16**. The high conversion observed may challenge the importance of the armed-disarmed donor concept under these specific flow glycosylation conditions, as donor **167** is certainly disarmed, but gives the same product conversion as armed model donor **62** under the same reaction conditions. However, given the disarmed nature of the donor, the temperature dependence of glycosylation reactions using donor **167** was probed in order to increase yield still further.

Table 16. *Temperature dependence of the glycosylation reaction using donor **167** and acceptor **155**.*



Entry	Temperature / ˚C	NMR Yield of Product 172 (%)
1	RT	85
2	0	81 (67)[a]
3	50	94
4	75	90

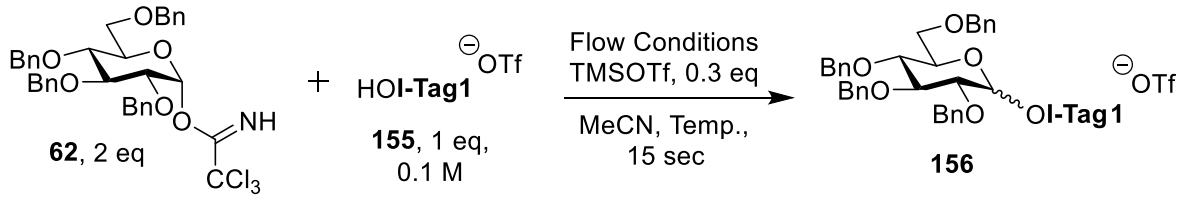
[a]Isolated yield.

Different temperatures were achieved by submerging the flow reactor in either an ice-water bath or an oil bath set at an elevated temperature. Entry 2 of **Table 16** reveals that performing the reaction at 0 ˚C is mildly detrimental to the reaction, giving 81 % conversion to product **172** compared to 85 % at RT. Conversely, elevating the temperature to 50 or 75 ˚C is beneficial to reaction progress (entries 3 and 4), with 94 % conversion at 50 ˚C being optimal for this reaction.

At this point, the model reaction between donor **62** and acceptor **155** was revisited to investigate temperature dependence in this system. The results are summarised in **Table 17**. In this case, both lowering and raising the temperature of the reaction (entries 2,3 and 4) appears to be marginally beneficial for the reaction, although the best result was obtained by lowering the temperature to 0 ˚C. By contrasting the experimental results of temperature dependence, the reactivity difference between the disarmed donor **167** and armed donor **62** is highlighted. It appears that in the case of **167**, raising reaction temperature helps to overcome the inherent lack of reactivity for this donor up to 50 ˚C. Raising the temperature still further to 75 ˚C may result in undesired side reactions that begin to cause a dip in conversion to the desired product. In the case of donor **62**, raising the temperature does seem

to boost reaction progress a little, but lowering the temperature may help to temper the high reactivity of the donor, resulting in the highest product conversion observed.

Table 17. *Temperature dependence of the glycosylation reaction using armed model donor* ***62*** *and acceptor* ***155****.*



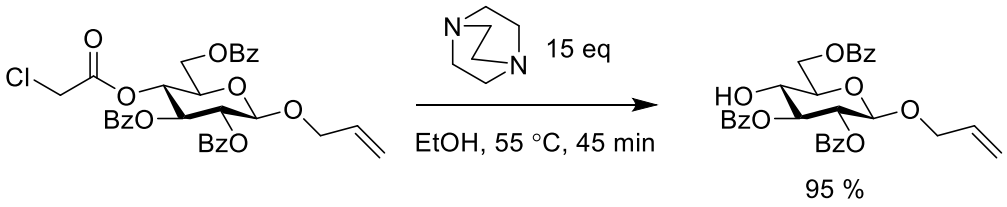
Entry	Temperature / °C	NMR Yield of Product 156 (%)
1	RT	85
2	0	94
3	50	90
4	75	90

With the optimal temperature for the reaction using donor **167** found, experiments could progress in order to try and build an oligosaccharide using this key structural motif.

4.6.6. Reactions Using a Chloroacetate Protected Donor

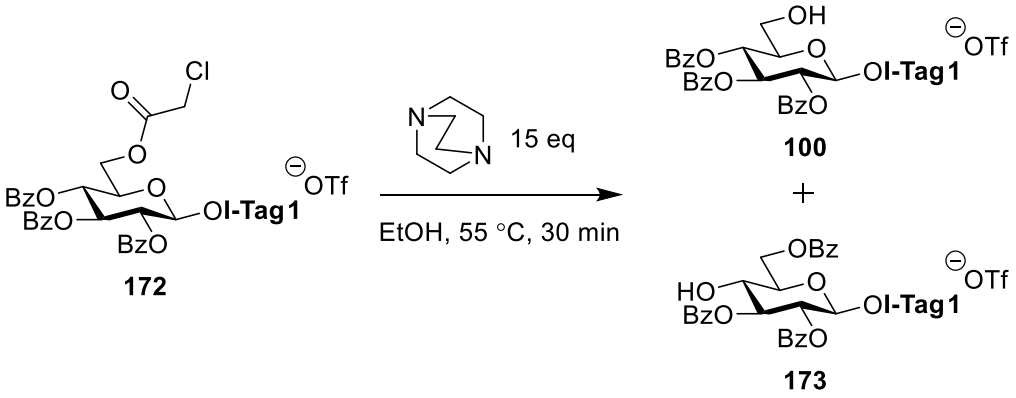
4.6.6.1. Deprotection Conditions

With I-Tagged product **172** in hand, the next step was to determine deprotection conditions for the chloroacetate group at C-6. Vliegenthart and co-workers reported the tertiary amine DABCO as a selective dechloroacetylation reagent (**Scheme 67**).[162] DABCO selectively cleaves the chloroacetate group on the substrate in the presence of benzoyl esters in just 45 min. Furthermore, no benzoyl neighbouring group migration is observed. This approach therefore seemed like an ideal deprotection strategy for the I-Tagged glycoside **172**.



Scheme 67. *Orthogonal dechloroacetylation reaction reported by Vliegenthart and co-workers.*[162]

Unfortunately, the dechloroacetylation reaction of **172** did not proceed as smoothly as expected (**Scheme 68**). By MS quantitative conversion of starting material to a single peak at m/z 615.0 was seen which suggested complete reaction. However, TLC showed two spots in the expected polarity region rather than one, whilst ^{1}H NMR spectroscopy revealed that neighbouring group migration had occurred. Under the basic reaction conditions, benzoyl migration from C-4 to C-6 took place rapidly, forming an inseparable mixture of the desired C-6 alcohol **100** and undesired C-4 alcohol **173**. Further experiments were performed adjusting stoichiometry of DABCO and solvent used, but neighbouring group migration was found to occur in all cases.



Scheme 68. *Dechloroacetylation using DABCO leading to an inseparable mixture of desired product* ***100*** *and neighbouring group migration product* ***173****.*

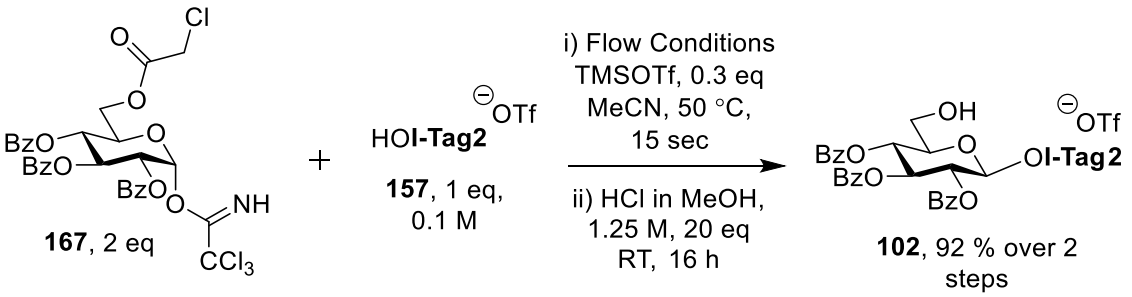
To overcome this problem, a deprotection strategy that did not use basic conditions was needed. Earlier in the project it was discovered that a methanolic solution of HCl partially cleaved the acetate group of I-Tagged glycoside **161** over 16 h (**Scheme 62**). It was reasoned that since the carbonyl group in a chloroacetate group is more electrophilic than the carbonyl group in an acetate group owing to the inductive electron withdrawal of the chlorine atom,

the chloroacetate group should be at least equally, if not more, susceptible to acid catalysed transesterification than the acetate group. To test this hypothesis, **172** was treated with 20 eq of a 0.8 M HCl solution in methanol. Pleasingly, after 3 h reaction, NMR spectroscopy revealed desired deprotected product **100** as the sole product (**Scheme 69**).

Scheme 69. *Acid catalysed transesterification of the chloroacetate group in **172** occurs selectively to give product **100** quantitatively.*

4.6.6.2. Flow-to-pot Glycosylation-Deprotection Strategy

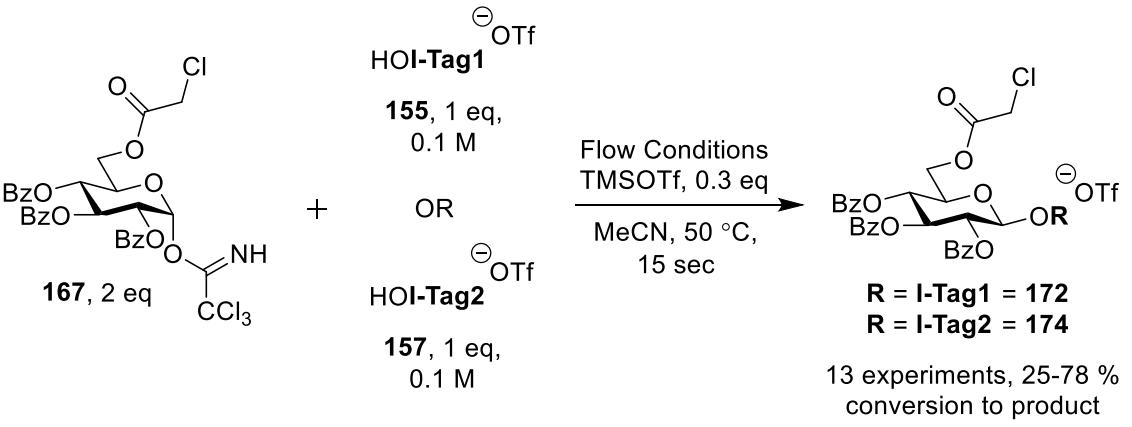
From this point onward, **I-Tag2** was preferentially used over **I-Tag1**, since the final step of the I-Tag supported oligosaccharide preparation would be I-Tag cleavage. This is much simpler with benzyl linked **I-Tag2** than with propyl linked **I-Tag1**. It was envisaged that a rapid and straightforward two step glycosylation-deprotection strategy may be performed using donor **167**. As had been previously done with the TIPS protected donors, the glycosylation solution leaving the flow reactor was fed directly into a methanolic HCl solution to facilitate immediate dechloroacetylation. Subsequent purification through solvent washing affords the pure I-Tagged saccharide product with a free C-6 hydroxyl group. Using this strategy, glycoside **102** was prepared in 92 % yield over two steps, as shown in **Scheme 70**. This was an excellent starting point from which to begin building an oligosaccharide chain.



Scheme 70. *Rapid glycosylation-deprotection-trituration strategy affords glycoside* ***102*** *in excellent yield over two steps.*

4.6.6.3. Irreproducibility of Reactions Using a Chloroacetate Protected Donor

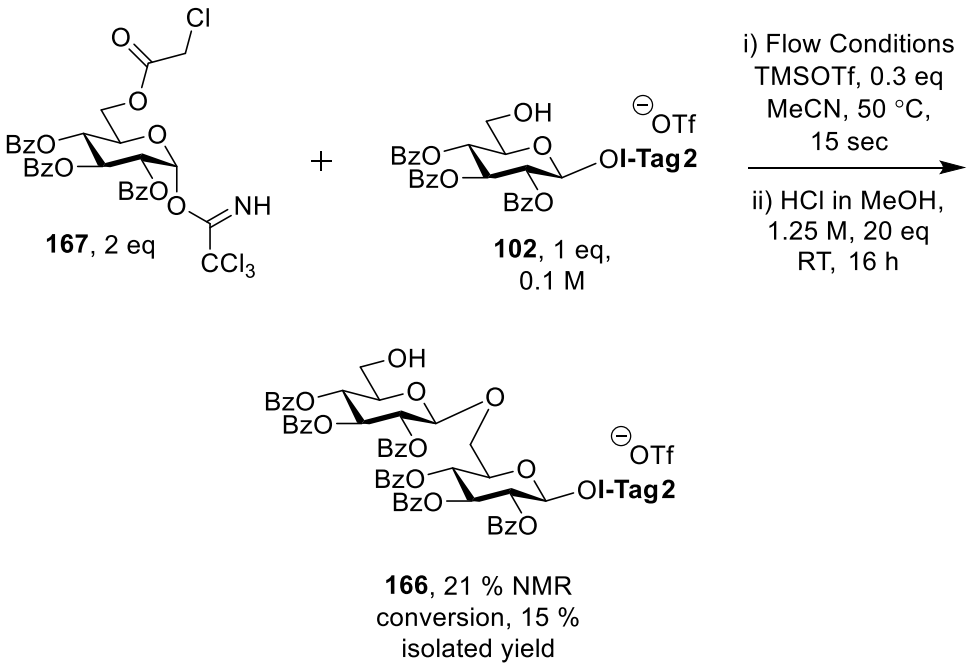
Excellent glycosylation results using donor **167** had been obtained. Frustratingly, these results were subsequently found to be irreproducible. Glycosylation experiments using either acceptor **155** or **157** were performed many times in an attempt to replicate the earlier results obtained (**Scheme 71**). A huge range of conversions to product were recorded by NMR spectroscopy, ranging from just 25 % at the lower end up to 78 % at the higher, though this is still substantially lower than the conversions of >90 % seen previously. The conversion to product percentage figure appeared to be almost random when performing consecutive experiments, with no clear pattern.



Scheme 71. *Highly inconsistent results were found when donor* ***167*** *was used for subsequent glycosylations.*

Initially, reaction conditions in the experiments described in **Scheme 71** were maintained precisely the same as the experiments that had been previously performed. When

these conditions failed to give the same high yields as they had previously, variables were altered to determine the cause of the problem. Purification of glycosyl acceptors **155** and **157** by reverse-phase HPLC and freeze-drying had no effect on glycosylations, suggesting that moisture or an impurity in the acceptor was not the issue. Distillation of TMSOTf and ensuring the use of very dry solvent similarly did not improve the reaction. The donor **167** was also dried thoroughly under high vacuum to remove any volatile impurities or solvents, including water, that may be preventing reaction from taking place. This measure also had no effect. Glycoside **102** was used as an acceptor with donor **167** to determine whether a different acceptor may help, however the result, shown in **Scheme 72**, was disappointing. ^{1}H NMR analysis of the crude material showed 21 % conversion of acceptor **102** to product disaccharide **166**. Disaccharide **166** was obtained in just 15 % isolated yield following HPLC purification.



Scheme 72. *Reaction of glycosyl acceptor **102** with donor **167** and subsequent dechloroacetylation gave disaccharide **166** in poor yield.*

Finally, all variables were maintained other than the donor in a control experiment. Using model perbenzylated donor **62** with acceptor **155** once again gave product **156** in 93 % conversion, as would be expected from previous results. This experiment confirmed that whatever issue was preventing glycosylation from occurring came from the donor **167**. Donor

167 had at this point been synthesised a total of three times, each by different synthetic routes. The first two quantities synthesised (the synthetic pathway for one of which is shown in **Scheme 66**) had worked well in the glycosylation. However, the third quantity prepared had never worked well. It was therefore assumed that whatever was preventing glycosylation from happening must be some impurity in the third quantity of donor **167**. However, by ^{1}H and ^{13}C NMR analysis, this quantity of donor was at least as pure as the first two, if not more so, as shown in **Figure 26**. The third quantity was subjected to column chromatography purification once again to try and remove any impurity that may be interfering with the glycosylation. Subsequent use of this purified quantity of donor **167** in a flow reaction proved the column chromatography to be ineffective, as conversion to product **174** was just 32 %. Next, the donor was dissolved in DCM and aqueous washes with water and brine were performed to remove any inorganic salt impurities in the donor sample that would not be visible through ^{1}H and ^{13}C NMR spectroscopy. Subsequent solvent removal and another glycosylation test proved that the aqueous wash had made no difference to the glycosylation outcome.

To overcome whatever problem there was with the third quantity of donor **167**, a fourth quantity was prepared following the synthetic route shown in **Scheme 66**, with the ^{1}H NMR spectrum shown in **Figure 26**. As the donor obtained through this route had worked well previously, it was hoped that another quantity of donor prepared this way would also perform well in glycosylations. However, when a glycosylation reaction was performed using the fourth quantity of donor **167** and acceptor **157**, a poor conversion to product **174** of approximately 47 % was seen, demonstrating that the synthesis route was not the cause of the problem.

Often, when purifying trichloroacetimidate donors by column chromatography, a small amount of triethylamine added to the elution solvents may help to prevent acid catalysed hydrolysis of the donor. This had been the purification strategy for most of the trichloroacetimidate donors synthesised by my hand, for example model donor **62**, and no issues had been encountered. But it was reasoned that if residual triethylamine, or salts thereof, were present in donor **167**, even in very small quantities, they may quench the TMSOTf and retard glycosylation. Thus, the fourth quantity of donor **167** was subjected to column chromatography once again, but this time with no triethylamine added. Testing the

freshly purified sample with acceptor **155** showed just 25 % conversion to product **172**. Analysis of the crude reaction mixture by MS and NMR spectroscopy revealed mostly hydrolysed donor **171**.

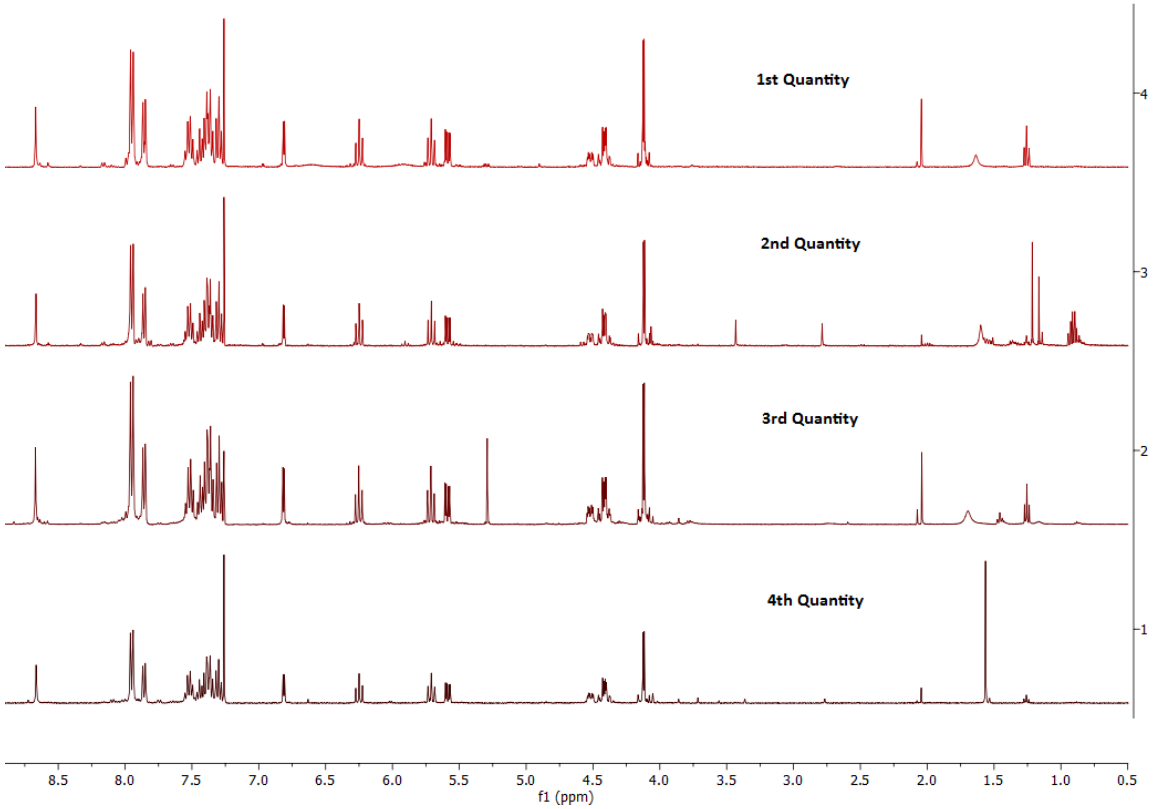
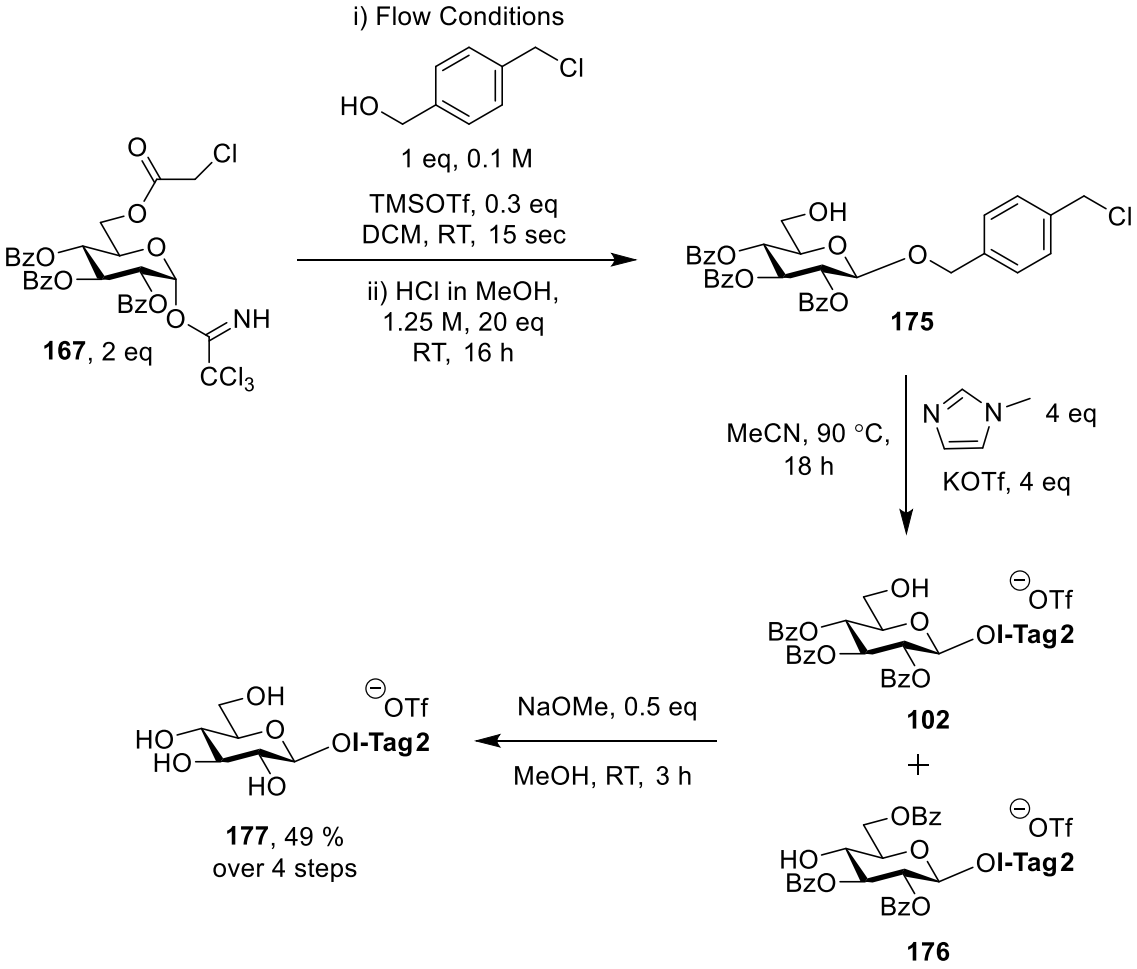


Figure 26. *Comparison of the ^{1}H NMR spectra of the four quantities of donor **167** synthesised. Using the 1st and 2nd quantities of donor **167** gave high yields of glycoside product, whilst using the 3rd and 4th quantities failed to replicate the previous high product yields.*

At this point, the list of possible reasons I (and my helpful lab colleagues) could imagine for reaction failure was exhausted. Hence, it was conceded that in this system, donor **167** was too inconsistent for use. However, there was one further experiment conducted using donor **167** that gave a further clue about the purity of the donor. Having a stock of donor **167** to hand, this donor was chosen as the starting material to prepare target molecule **177**. The synthesis is shown in **Scheme 73**. 4-(Chloromethyl)benzyl alcohol was glycosylated with donor **167** under the standard flow glycosylation conditions. ^{1}H NMR spectroscopic analysis at this point proved there was no acceptor remaining in the crude product, suggesting it had all been consumed in the reaction. Deprotection of the chloroacetate group was then

performed using methanolic HCl as previously described. Next, treatment with 1-methylimidazole and potassium triflate generates **I-Tag2** through nucleophilic substitution of the chlorine atom, but also causes partial neighbouring group migration of the C-4 benzoyl group to C-6, owing to the basicity of 1-methylimidazole. This gives a mixture of I-Tagged products **102** and **176**. The mixture is then treated with a sodium methoxide solution to remove all benzoyl groups over 3 h. Thus, desired compound **177** is furnished in 49 % yield over four steps.



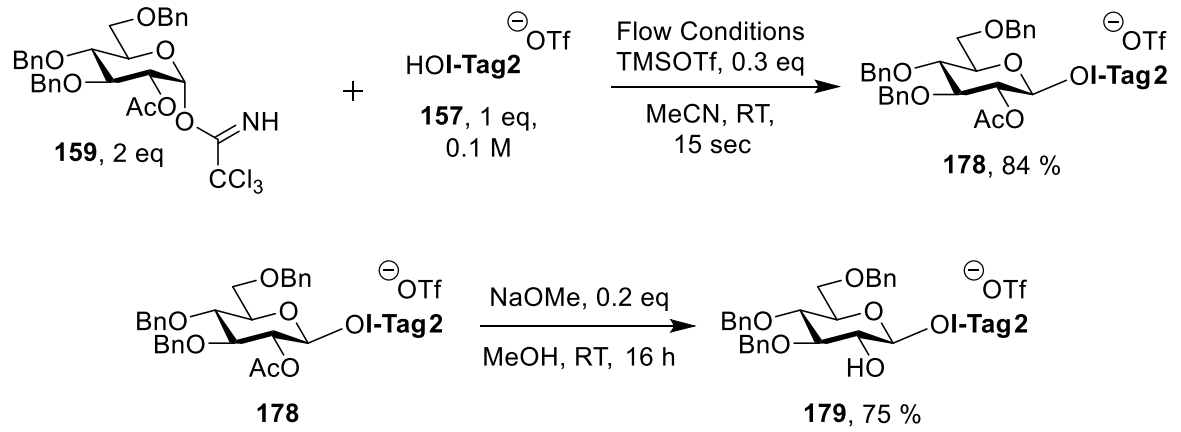
Scheme 73. *Synthetic route from donor **167** to I-Tagged glucose **177**.*

All evidence acquired thus far indicated that some impurity present in the later quantities of donor **167** was responsible for the poor yields acquired. However, the smooth glycosylation of donor **167** with neutral acceptor 4-(chloromethyl)benzyl alcohol indicates that this is unlikely. Two possible explanations for the observed irreproducibility were

hypothesised. Firstly, there may have been some impurity present in the first two quantities of donor synthesised that actually helped the glycosylation with I-Tagged acceptors to proceed in high yields. Hence, the lack of this beneficial impurity in later quantities prevented such complete glycosylation from occurring. Secondly, since I-Tagged glycosyl acceptors have very highly polarised ionic charges in close proximity to the reacting alcohol group, one might expect the nucleophilicity of that alcohol group to decrease through a variety of factors, perhaps including inductive electronic components and the influence of the solvation shell. Therefore, since the I-Tagged glycosyl acceptors suffer from poor nucleophilicity, inconsistent glycosylation results might be expected. Conversely, neutral glycosyl acceptors such as 4-(chloromethyl)benzyl alcohol are more nucleophilic and hence higher glycosylation yields are seen when these acceptors are used.

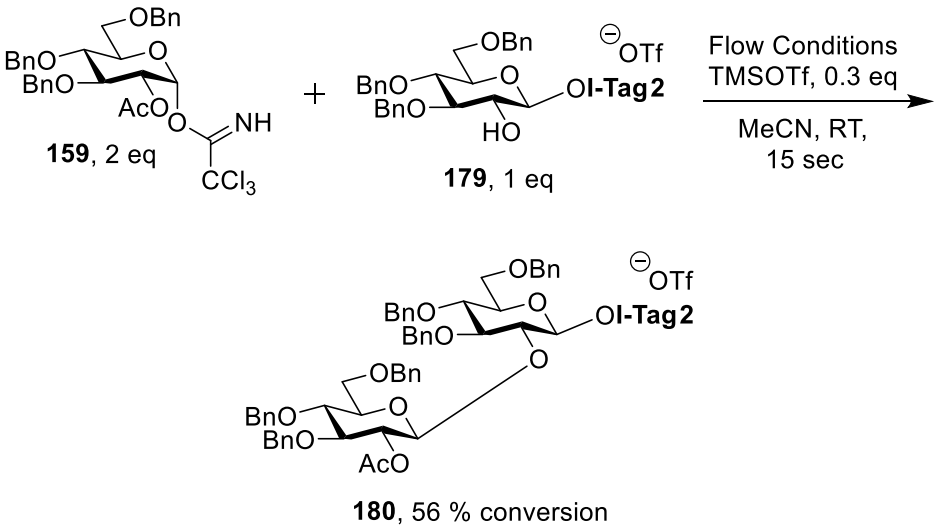
4.6.7. **Synthesis of β-1,2-Glucans Using a Super-Armed Donor**

As a result of the inconsistent results acquired using donors with an orthogonal C-6 protecting group, the construction of β-1,2-glucans using donor **159** that had given positive results in glycosylation experiments thus far was pursued. It was reasoned that synthesising β-1,2-glucans could showcase the utility of the I-Tag supported flow glycosylation method just as well as synthesising β-1,6-glucans. The C-2 acetate group present on donor **159** allows complete β selectivity in glycosidic bond formation and subsequent deprotection using sodium methoxide would liberate a free C-2 alcohol group that may then be used as a glycosyl acceptor. Thus, the reaction between donor **159** and acceptor **157** gave glycoside **178** in quantitative conversion by ^{1}H NMR spectroscopy. Purification by trituration gave **178** in 84 % isolated yield (**Scheme 74**). Subsequent acetate removal gives free alcohol **179** in good yield.



Scheme 74. *Glycosylation of donor **159** with acceptor **157** gives I-Tagged sugar **178** in excellent yield. Subsequent acetate deprotection liberates glycosyl acceptor **179**.*

Monosaccharide **179** was then used in a glycosylation reaction using the standard optimised flow conditions. It was hoped that the reaction would proceed in high yield, however, acceptor **179** bears a secondary alcohol. Secondary alcohols are more sterically hindered than primary alcohols, and thus it is common to see reduced reactivity of secondary alcohols in glycosylations. Often longer reaction times or elevated temperatures are used to force secondary alcohols to undergo complete reaction. Realising these concerns, disaccharide **180** was synthesised in 56 % conversion from acceptor **179** as shown in **Scheme 75**. This partial conversion from acceptor to product causes difficulty during purification. Highly polar I-Tagged acceptors **155** and **157** are water soluble, and thus any unreacted acceptor may be separated from the I-Tagged glycoside product through an aqueous wash. In this case, both acceptor **179** and product **180** are saccharide-derived I-Tagged molecules and thus their separation by simple solvent washes is impossible. However, they can be separated by HPLC or size-exclusion chromatography. Thus, it was decided to take advantage of this partial glycosylation to prepare different oligosaccharide fragments in one pot and do a single chromatographic purification at the end of the synthesis to separate the mixture of oligosaccharides in a manner similar to that described in the prior publication concerning combinatorial ICROS.[146]

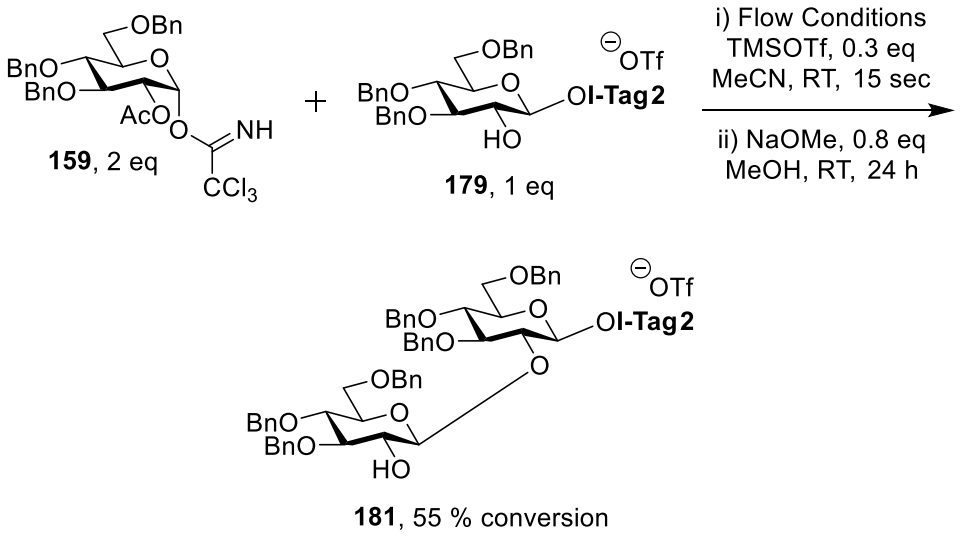


Scheme 75. *Reaction of donor **159** and acceptor **179** gives disaccharide **180** in 56 % conversion.*

The next step was thus to take the 44:56 ratio mixture of **179**:**180** and treat with sodium methoxide to remove the acetate group from **180** to furnish disaccharide acceptor **181**. Reaction progress was monitored by MS, showing complete acetate removal over 24 h using 0.8 eq of sodium methoxide. However, after workup and trituration, a very low mass of products was recorded, much lower than what would be expected from the mass of reactants. Furthermore, the ratio of monosaccharide to disaccharide had changed substantially from that observed immediately after glycosylation. The reason for this was the neutralisation method. After complete acetate removal, the pH of the solution was brought to 7. In this case, neutralisation was achieved using Amberlite acidic resin, which bears surface sulfonic acid groups. It became evident that these resin-bound sulfonic acid groups, after proton donation, would become sulfonate anionic residues that require a cation to balance the charges. Since the I-Tagged products are themselves cationic, they could bind to the solid resin, resulting in the observed low product mass after solution is separated from the resin beads. Hence, the oligosaccharide synthesis was repeated, with sodium methoxide neutralisation achieved with aqueous hydrochloric acid solution.

The second synthesis of monosaccharide acceptor **179** was achieved in the same manner as previously, however, fully protected glycoside **178** was not purified prior to basic acetate deprotection. Instead, solvent was removed from the crude glycosylation mixture, then the residue was dissolved in sodium methoxide solution directly. Subsequent

purification gave product **179** in 72 % yield over two steps, slightly higher than the 63 % yield over two steps reported in **Scheme 74**. In the same manner, acceptor **179** was transformed into product **181** as shown in **Scheme 76**. In close agreement with the result found previously in **Scheme 75**, 55 % conversion from **179** to **181** was seen by ^{1}H NMR spectroscopic analysis. Given this 45:55 ratio of **179**:**181**, an expected combined mass could be calculated based upon the number of moles of acceptor **179** used for the reaction. The "yield" of the product mixture was determined to be 76 % over two steps. Presumably the reason for loss of products is due to minor amounts of I-Tagged material being dissolved in the trituration solvent washes and mechanical losses during workup steps such as drying the organic solution with magnesium sulfate desiccant and subsequent filtration.



Scheme 76. *Glycosylation using acceptor **179** and immediate deprotection gives disaccharide **181**.*

The next step was to subject the mixture of mono- and disaccharide acceptors **179** and **181** to the flow glycosylation conditions using donor **159** once again to synthesise a trisaccharide, whilst increasing the proportion of disaccharide present in the product mixture. At this point, since the ^{1}H NMR spectra contained too many overlapping multiplets to be of use for characterising the product mixture, mass spectra were relied upon exclusively, using the inherently strong signal intensity for I-Tagged compounds to monitor the reaction. Note that reliable quantitative information cannot be extracted from these mass spectra, however, they are nonetheless useful for determining which species are present with some approximation as to quantity based on peak height, since molar amount and peak height are

positively correlated. Immediately following the glycosylation reaction, the ESI mass spectrum appeared as shown in **Figure 27**. Peaks at *m/z* 635 and 1067 correspond to unreacted acceptors **179** and **181**. The peak at *m/z* 1109 corresponds to disaccharide **180**, formed by reaction of acceptor **179** with a molecule of donor. Likewise, trisaccharide **182**, formed by reaction of disaccharide acceptor **181** with a molecule of donor, is responsible for the peak at *m/z* 1541. Note that the negative ion mass spectrum simply shows a very large peak at *m/z* 148.8, corresponding to the triflate counterion common to all the I-tagged cations.

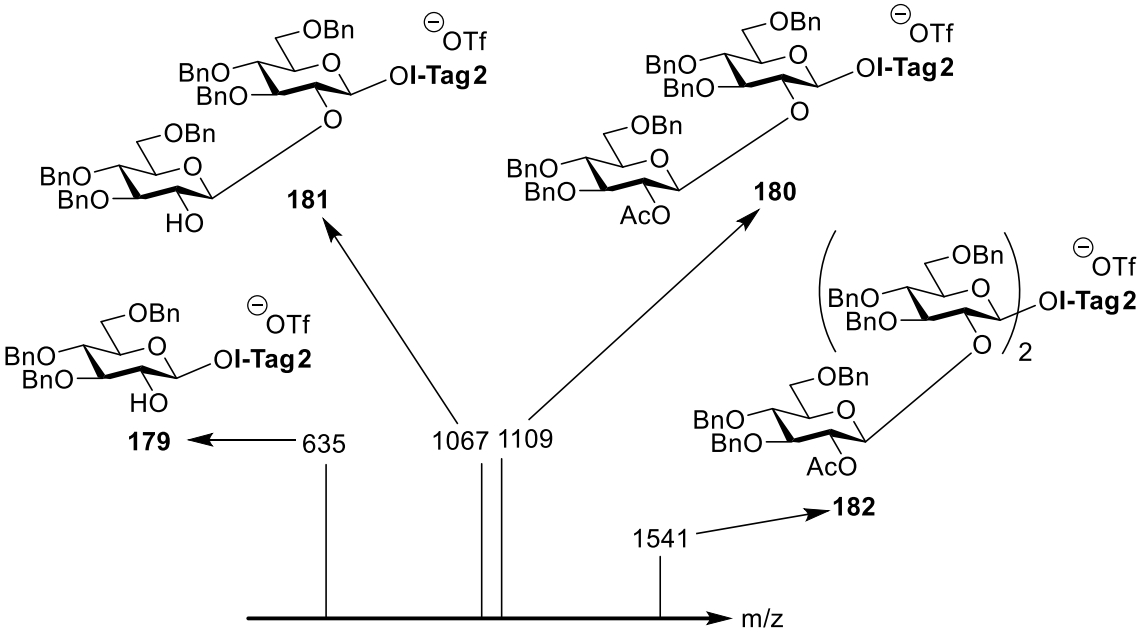


Figure 27. *ESI mass spectrum of the crude reaction mixture acquired following the glycosylation reaction using the **179/181** glycosyl acceptor mixture. m/z values for the signals are labelled, with the causative chemical species shown by an arrow from the corresponding m/z value.*

As deacetylation began, it was noted that acetate deprotection of trisaccharide **182** was significantly slower than disaccharide **180**. To speed up the reaction, a total of 2.75 eq of NaOMe were added over 48 h to achieve complete reaction. Mass spectrometry showed the coalescence of the two disaccharide peaks into a single peak at 1067 as **180** transforms to **181**, whilst the trisaccharide peak had a value of 1499 corresponding to product **183** following complete deprotection. Moving forward, the mono-/di-/trisaccharide mixture was glycosylated once again with donor **159** to make a tetrasaccharide, and the product mixture

was treated with sodium methoxide. The deprotection of disaccharide **180** and trisaccharide **182** was complete within 19 h using 2.5 eq NaOMe, however, the tetrasaccharide was very resistant to acetate deprotection, even when a large (>20 eq) amount of sodium methoxide was added complete deprotection could not be achieved. As a result, it was reasoned that the tetrasaccharide would be a sensible maximal oligosaccharide length for this synthesis, since smaller oligosaccharides could be easily deprotected in the presence of protected tetrasaccharide. Thus, further glycosylations might incrementally enrich the proportion of longer oligosaccharides up to tetrasaccharide by partially consuming the mono-, di- and trisaccharides acceptors in each glycosylation reaction. Unfortunately, due to time constraints, no further glycosylations were carried out on this product mixture and the constituent oligosaccharides were not separated. Thus, a drawing of the final mass spectrum acquired is shown in **Figure 28**.

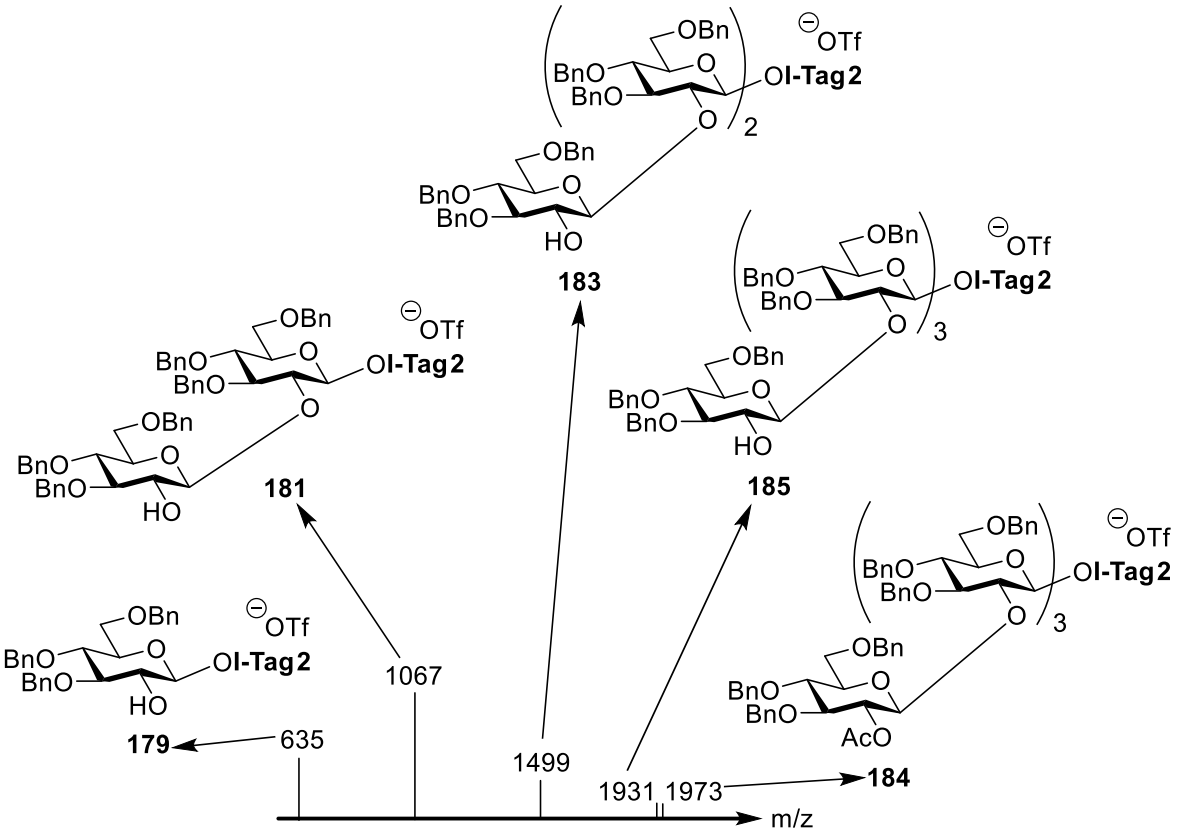
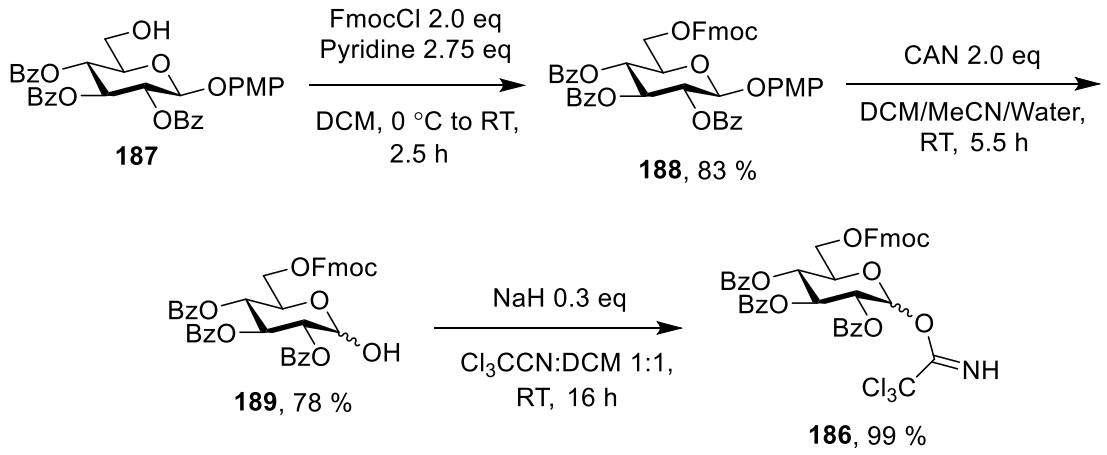


Figure 28. *ESI mass spectrum of the final β-1,2-glucan saccharide mixture synthesised.*

4.6.8. **Fmoc Protected Donors for β-1,6-Glucan Preparation**

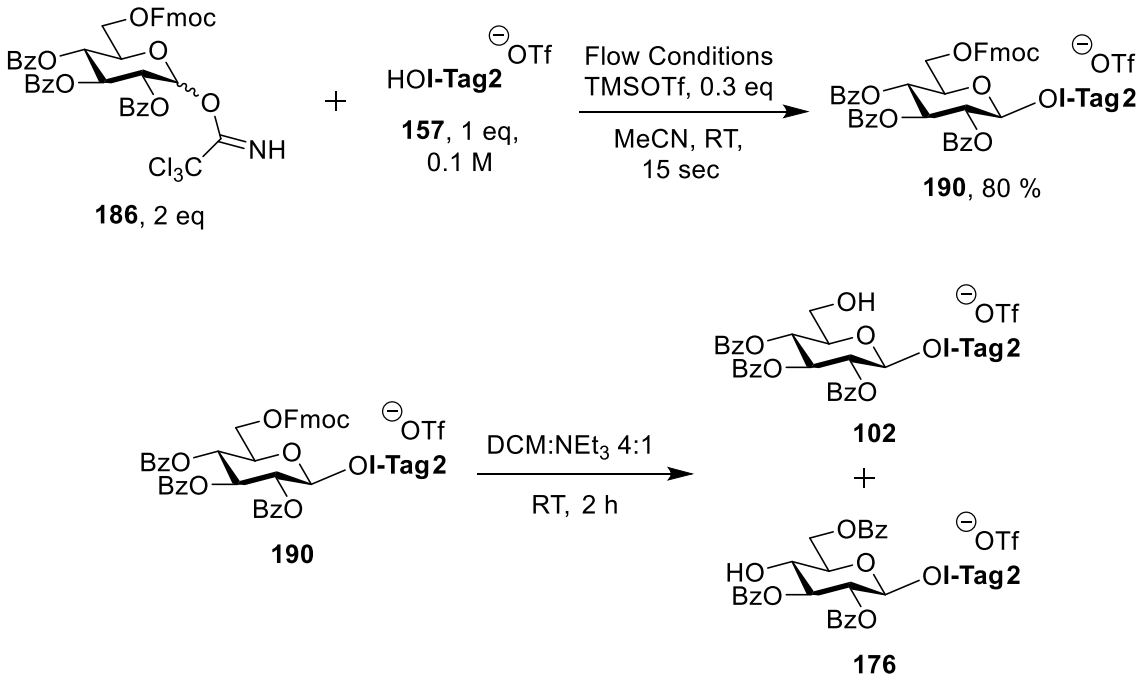
Since the β-1,2-glucan synthesis gave only moderate conversions once secondary acceptors were used, the construction of β-1,6-glucans that used primary alcohol acceptors exclusively was revisited once again. Earlier work had proven that several C-6 orthogonal protecting groups including silyl ethers and chloroacetate groups were unsuitable, however, one protecting group that had not yet been considered was the 9-fluorenylmethoxycarbonyl (Fmoc) group. The Fmoc group is commonly used in peptide synthesis to protect amines as a carbamate,[163] but upon reaction with an alcohol rather than an amine 9-fluorenylmethoxycarbonyl chloride forms a carbonate group that acts as a base labile protecting group.

Previous work by Boons and co-workers[164] showed that the Fmoc protecting group could be installed on a carbohydrate alcohol group and tolerates other protecting group manipulations around the molecule. One potential stumbling block is the introduction of the acetimidate group for the formation of a trichloroacetimidate donor, since this reaction generally requires catalysis by an organic base such as DBU that is also likely to cleave the Fmoc group. However, the authors found that using sodium hydride as the base allowed installation of the trichloroacetimidate group whilst leaving the Fmoc protecting group intact. Moreover, the Boons group used Fmoc-protected trichloroacetimidate donors in high yielding glycosylations with TMSOTf as the promoter. Finally, Fmoc deprotection using triethylamine in DCM was demonstrated to proceed in high yield. This combination of factors made the Fmoc group appear to be a suitable C-6 protecting group choice for my system. One concern was that the basic deprotection conditions may result in undesired neighbouring group migration in the manner caused by DABCO as shown in **Scheme 68**. Nonetheless, donor **186** was synthesised as shown in **Scheme 77**. Previously synthesised intermediate **187** was chosen as the starting material. Treatment with FmocCl furnishes fully protected **188** in 83 % yield. Subsequent deprotection of the anomeric *para*-methoxyphenyl group using CAN gives hemiacetal **189** in 78 % yield. Finally, sodium hydride mediated introduction of the trichloroacetimidate group gives donor **186** as a mixture of anomers, however, only the α anomer could be completely separated by column chromatography.



Scheme 77. *Synthesis of orthogonally protected donor* ***186*** *bearing an Fmoc group from intermediate* ***187****.*

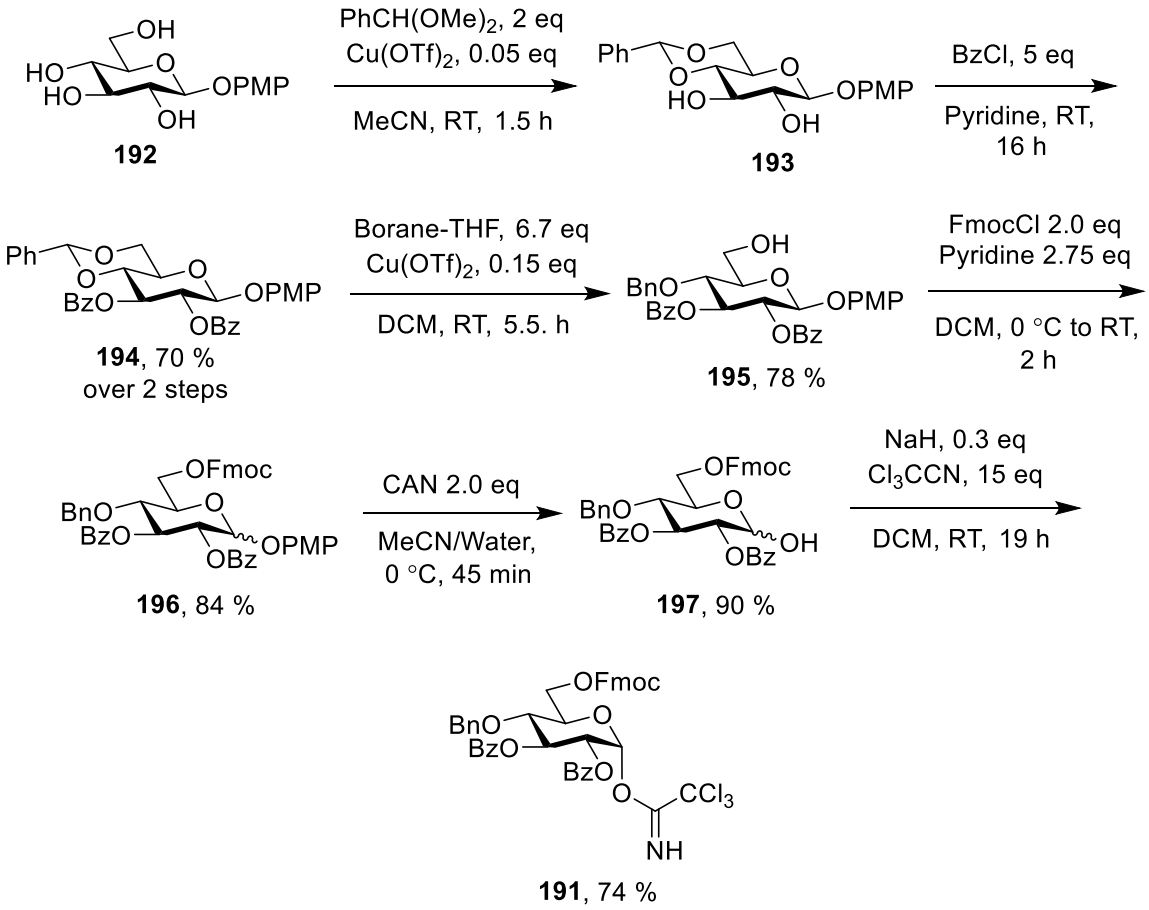
Using donor **186** in a flow glycosylation with acceptor **157** gave I-Tagged glycoside **190** in 80 % isolated yield using the optimised glycosylation conditions, as shown in **Scheme 78**.



Scheme 78. *Glycosylation of acceptor* ***157*** *with donor* ***186*** *furnishes product* ***190*** *in good yield. Subsequent basic Fmoc deprotection results in C-4 benzoyl group migration to C-6.*

However, the ensuing basic Fmoc deprotection caused the C-4 benzoyl group to partially migrate to C-6, resulting in an inseparable mixture of desired compound **102** and

migration product **176**. This lamentable result cemented the intolerance of benzoyl protected glycoside **102** to basic conditions. Nevertheless, the synthesis of donor **186** and its subsequent high yielding glycosylation proved that the Fmoc protecting group was well-suited to the glycosylation protocol. It was reasoned that since there is no evidence of migration of the benzoyl groups at C-2 or C-3, changing the C-4 protecting group to one that is incapable of migration, whilst maintaining the other protecting groups, ought to result in a viable protecting group pattern. Thus, 4-*O*-benzyl protected donor **191** was synthesised as shown in **Scheme 79**.



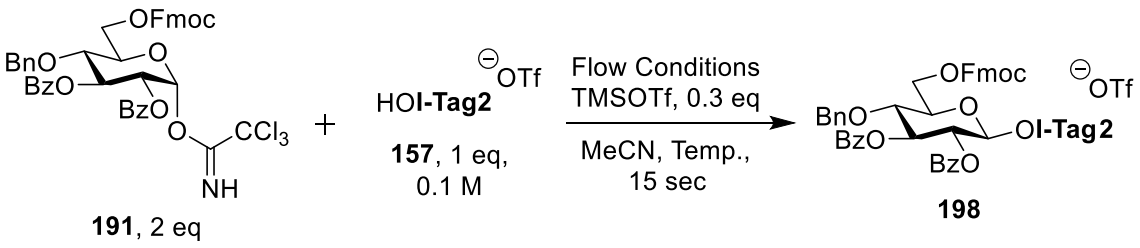
Scheme 79. *Synthetic route from starting material **192** to donor **191**.*

The synthesis begins with anomerically protected starting material **192**. Copper(II) triflate catalysed benzylidenation gives **193**, with perbenzoylation of this compound giving fully protected **194** in 70 % yield over two steps. Next, regioselective benzylidene reduction using borane-THF and copper(II) triflate installs a benzyl group at C-4 selectively to give **195** in 78 % yield. Introduction of the Fmoc group at C-6 proceeded in good yield, although caused

unexpected anomerisation. *para*-Methoxyphenyl removal with CAN gives hemiacetal **197** in excellent yield. Finally, sodium hydride mediated installation of the trichloroacetimidate group initially produced an anomeric mixture, as judged by TLC and TLC-MS. However, allowing the reaction to equilibrate over 19 h results in anomerisation to the α anomer exclusively. Thus, donor **191** was prepared in 74 % yield as a single anomer.

When donor **191** was glycosylated with acceptor **157** at room temperature (entry 1, **Table 18**), an excellent conversion to product **198** of 87 % was seen by ^{1}H NMR spectroscopy. In order to optimise temperature for glycosylation of this donor, other glycosylation experiments were performed at 0 ˚C, 50 ˚C and 75 ˚C (entries 2-4). Intriguingly, altering temperature makes virtually no difference to product conversion, so room temperature was the best option for pragmatic reaction setup reasons.

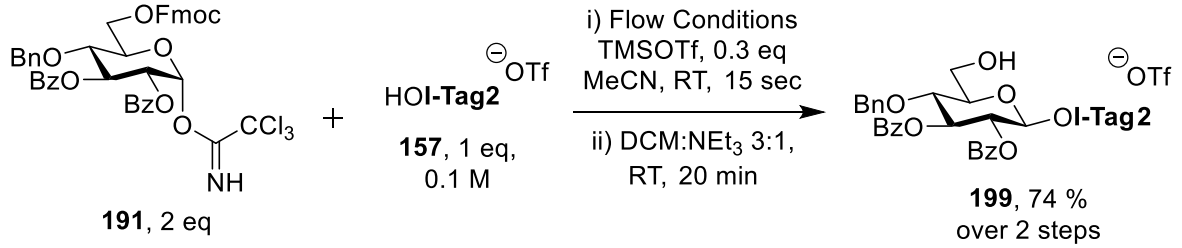
Table 18. *Temperature dependence of the glycosylation reaction using donor **191** and acceptor **157**.*



Entry	Temperature / ˚C	NMR Yield of Product 198 (%)
1	RT	87
2	0	90
3	50	87
4	75	88

Crucially, basic deprotection of **198** with triethylamine occurs quantitatively as judged by MS, whilst NMR spectroscopy and TLC show only desired product **199** with no migration side products. Applying the glycosylation and basic deprotection together in a flow-to-pot strategy as shown in **Scheme 80** furnishes product **199** in 74 % yield over two steps after purification. A workup procedure was developed for this system in order to allow complete

purification whilst minimising product losses to the aqueous phase. The DCM/NEt_3 mixture is diluted with 1 M $HCl_{(aq.)}$ and more DCM in order to protonate the triethylamine and extract it into the aqueous phase, whilst the product remains in the DCM phase. Washing the DCM phase with a portion of water removes lingering highly polar impurities. The aqueous washings are combined and washed with a further portion of DCM, which is then combined with the original DCM phase. This is done because the I-Tagged product **199** is polar enough that it may dissolve partially in the aqueous phase and thus an organic wash helps to extract any product in the aqueous phase back to the organic. This acidic aqueous phase in then discarded. Next, the DCM phase is washed with $NaHCO_{3\ (sat.\ aq.)}$ to deprotonate any final traces of triethylammonium cation, and a further water wash is performed. Once again, washing the combined aqueous portions with DCM and combining DCM washes maintains the maximal amount of product in the organic phase. Finally, the DCM phase is dried with magnesium sulfate, filtered and the solvent is removed under reduced pressure. This aqueous workup approach was required because ^1H NMR spectra revealed triethylammonium salt impurities in the product mixture when only an acidic wash was performed, whereas performing an acidic wash followed by a basic wash avoids this problem. Subsequent washes with hexane/Et_2O and drying under vacuum gives pure product **199**.



***Scheme 80**. Flow-to-pot glycosylation-deprotection using donor **193** and acceptor **159** furnishes product **199** in very good yield.*

At this stage of the project, a very fast flow-to-pot glycosylation-deprotection strategy has been developed using donor **191**. This furnishes **199**, a glycosyl acceptor that ought to serve as an excellent I-Tag supported scaffold for chain elongation and hence β-1,6-glucan synthesis.

5. Conclusions and Outlook

The studies performed during this PhD project represent a contribution to overcoming the impediments to efficient oligosaccharide construction laid out in the project aims through several avenues. Firstly, throughout each of the projects a series of glycosyl acceptors and donors were synthesised on a multi-gram scale. These include primary and secondary alcohol acceptors, including some that bear an I-Tag support. Donors prepared include glucals and galactals and several glucose-type trichloroacetimidate donors. Most of these donors and acceptors were prepared via multiple step syntheses in which rationally selected protecting groups were manipulated to control the steric and electronic properties of the donor. For certain novel compounds, for instance trichloroacetimidate donors **160**, **167**, **186** and **191** (**Figure 29**) bespoke routes to the target molecules were developed and optimised.

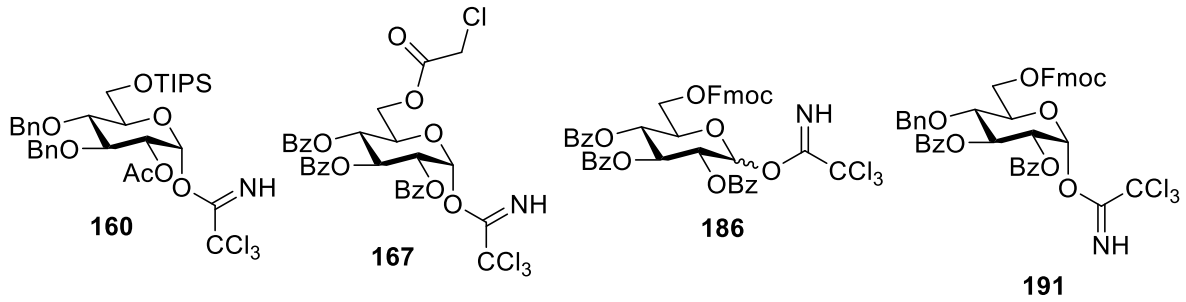


Figure 29. *Bespoke synthetic pathways to novel trichloroacetimidate donors **160**, **167**, **186** and **191** were developed and optimised.*

Next, organocatalytic glycosylation reactions were screened. Triazolium salt **128** was tested for its ability to catalyse 2-deoxyglycoside synthesis from a galactal donor, either alone or synergistically with thiourea **86**. Poor product conversion was observed, though this may be rationalised by a consideration of the pK_a values of the different species. Further work by colleagues identified thiourea **86** and BINOL-derived phosphoric acid **129** as dual organocatalysts for the efficient, high yielding synthesis of α 2-deoxyglycosides. To better understand the reaction mechanism, an experiment using methanol-d_4 was performed. A mixture of two isotopically labelled products **130** and **131** was obtained from the reaction and thus valuable mechanistic information about the glycosylation reaction was accrued.

Glycal activation via transition metal catalysis was concurrently explored as a strategy for 2-deoxyglycoside preparation. To that end, palladium catalysis was investigated. Following a screen of palladium catalysts and phosphine ligands conducted by colleagues, optimisation of the reaction conditions was carried out for model substrates using Pd^{II} catalyst **113** and phosphine ligand **114**. Conditions were met that gave 99 % conversion (and subsequent 86 % isolated yield) of acceptor alcohol **41** to product 2-deoxy-disaccharide **44**, with total α selectivity. Using the optimised reaction conditions, a series of primary and secondary alcohols were screened for compatibility with the glycosylation protocol. In general, excellent yields and α selectivity were obtained for primary alcohol acceptors in reasonable and convenient overnight reaction times of 17-23 h. Secondary alcohol acceptors proved more problematic; however, good yields were obtained for saccharide-derived glycosyl acceptors. Secondary general aliphatic and aromatic alcohols should, in general, be avoided as substrates in this methodology, owing to poor product formation and α/β selectivity. Furthermore, a probable reaction mechanism is discussed in light of mechanistic experimental data.

Ultimately, two novel catalytic strategies for 2-deoxyglycoside preparation were developed. The broad substrate scope and versatility of these reactions was explored and mechanistic information was collected, culminating in the publication of the findings. However, there is significant scope for further work in this area. Palladium is a rather expensive and scarce transition metal. An attractive alternative would be a metal catalyst that is cheaper and more abundant than palladium, but one that is also able to catalyse stereoselective 2-deoxyglycoside synthesis. Moreover, in the optimised conditions for the glycosylation protocol developed, 0.3 eq of palladium complex **113** is required for each reaction. This is a high catalyst loading that may limit the green credentials of this glycosylation strategy, since a significant amount of metal waste is produced in each reaction. It would be preferable to identify a metal catalyst or organocatalyst that permits rapid 2-deoxyglycoside synthesis at low catalyst loading, thus maximising the environmental and economic benefits of catalysis.

Another key issue that could be overcome in the future is the stereochemical outcome of the glycosylation reaction. Both catalytic 2-deoxyglycoside preparations discussed in this thesis favour α selectivity in the product. Ideally, a catalyst would be discovered that produces

the β anomer preferentially, overcoming the inherent bias for formation of the α anomer. Access to either the α or β 2-deoxyglycoside anomer selectively from a common glycosyl donor through complementary catalytic glycosylation approaches would represent an exceptionally useful tool in the glycoscientist's synthetic toolbox.

Moving forward, the gold catalysed synthesis of 2-deoxyglycosides in flow was explored, but little success was achieved. Several of the promising early results using benzoyl protected glycosyl acceptor **74** proved to be unique to that compound, since other acceptors performed poorly. What is clear is that the catalytic system is sensitive to impurities, even when repeating batch reactions. The identity of these impurities is not clear, due to inconsistent results that were very difficult to interpret. Overall, it was discovered that this reaction protocol is not well-suited to continuous flow methodology, despite similar gold catalysed glycosylations being performed in flow in the literature. Nonetheless, this was useful information to gather and the limits of the approach were found.

Finally, my attention was turned towards the glycosylation of I-Tagged sugars in flow, with some excellent results being obtained in this area. Optimisation of the model reaction between perbenzylated donor **62** and I-Tagged alcohol **155** was conducted, during which the residence time, concentration, stoichiometry of TMSOTf and donor and reaction temperature were all altered and optimised. In the best conditions, product **156** was formed in 94 % conversion in just 15 seconds. Thus, previously performed lengthy overnight glycosylations in batch were successfully shortened to a matter of seconds in flow under exceptionally mild and practical conditions. Furthermore, the rapid and simple trituration purification method minimises the volume of volatile organic solvents required which is environmentally beneficial as well as being cheaper. One can envisage the entire glycosylation-deprotection-trituration sequence being performed over the course of just a few hours, enabling extremely quick and facile oligosaccharide assembly.

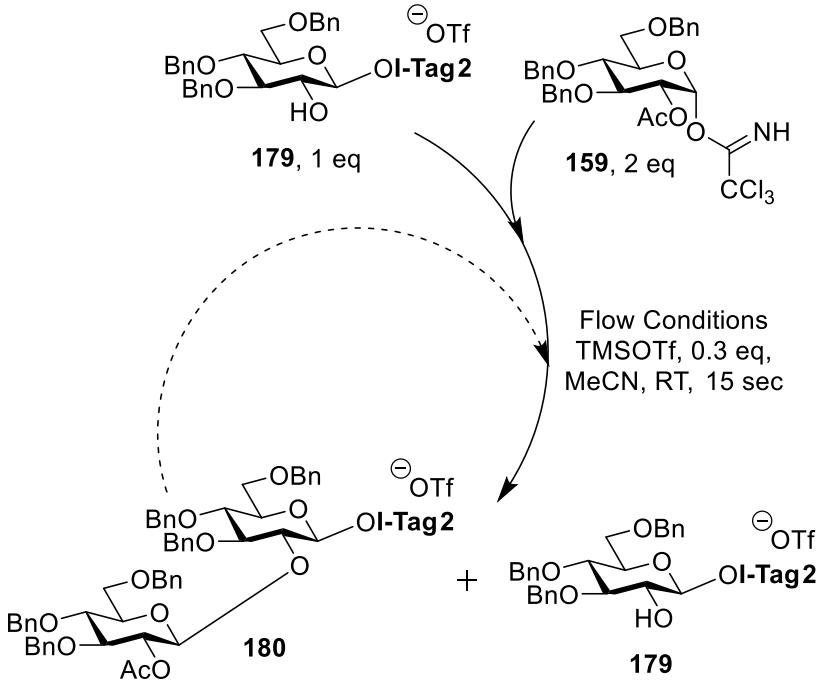
Substrate scope was then explored, in particular with respect to silyl ether protected donor **97**. Batch reactions using this donor could not be replicated in flow due to unexpected reactivity of the silyl ether group, including partial deprotection during the course of the glycosylation and intramolecular cyclisation of the donor to form 1,6-anhydro sugar **163**. Changing solvent, temperature or any other variable did not prevent this reaction pathway

and thus it seems that the observed reactivity is a feature of the flow regime. The use of an ethereal HCl solution for silyl ether deprotection is noteworthy, as it is much faster than a methanolic HCl solution and does not cause unwanted acetate group deprotection.

Chloroacetate protected donor **167** was synthesised as an alternative to a silyl ether protected donor. Initial results showed excellent conversion to products using the optimised conditions, whilst acid catalysed transesterification was found to be a good deprotection strategy that avoided benzoyl group migration. Subsequent experiments showed that the initial results were irreproducible, despite efforts to recover previously observed reactivity. The reasons for the irreproducibility are speculated upon in light of the successful reaction of donor **167** with 4-(chloromethyl)benzyl alcohol. Avoiding this donor for future experiments would be prudent to avoid inconsistent results.

The synthesis of β-1,2-glucans using super-armed donor **159** was also explored. Secondary alcohol acceptor **179** was synthesised in 72 % yield over two steps. Using acceptor **179**, moderate conversion to product disaccharide **180** of 56 % was found. Repeated glycosylations produced a mixture of mono-, di-, tri- and tetrasaccharides through an iterative glycosylation-deprotection-trituration sequence, with reaction progress monitored by mass spectrometry. In the immediate future, this mixture could be separated by chromatography to give a small library of different oligosaccharides.

There may be more simple and efficient ways to prepare β-1,2-glucans using this I-Tag supported flow glycosylation strategy that could be incorporated into future work. Firstly, the reaction of secondary glycosyl acceptors in the flow glycosylation system was not optimised, since acceptor **155** used for reaction optimisation was a primary alcohol. It may be the case that re-optimising the flow glycosylation reaction for secondary acceptors by altering variables such as stoichiometry of donor and TMSOTf, temperature and residence time will allow more complete conversion than the 55 % seen in **Scheme 75** and **Scheme 76**. Secondly, if complete conversion in the glycosylation is not observed, further glycosylations on the product mixture with no deprotection step may be useful, as shown in **Scheme 81**.



Scheme 81. *Suggested strategy of re-subjecting reaction mixture to glycosylation conditions in order to encourage complete conversion of acceptor **179** to product **180**.*

The first flow glycosylation gives 55 % conversion of **179** to **180** as previously. However, at this point, the mixture of fully protected disaccharide product and unreacted acceptor can be dried, combined with another 2 eq of donor **159** and re-subjected to the flow reaction conditions, causing further conversion of **179** to **180**. In this manner, minimal purification is needed, whilst conversion to product **180** can be maximised. If needed several glycosylation runs could be performed to achieve complete conversion of the glycosyl acceptor to product. Under normal circumstances, such an approach might be very time consuming, however, owing to the extremely short reaction times required for these flow glycosylations and rapid reaction analysis due to the I-Tag moiety, such a strategy becomes an expedient option for oligosaccharide synthesis.

Towards the end of the PhD project, Fmoc protected donors were explored as a route to make β-1,6-glucans. Whilst donor **186** bearing benzoyl protecting groups at C-2,3 and 4 undergoes smooth glycosylation, basic deprotection results in neighbouring group migration to produce an inseparable mixture of compounds. However, donor **191** featuring a C-4 benzyl

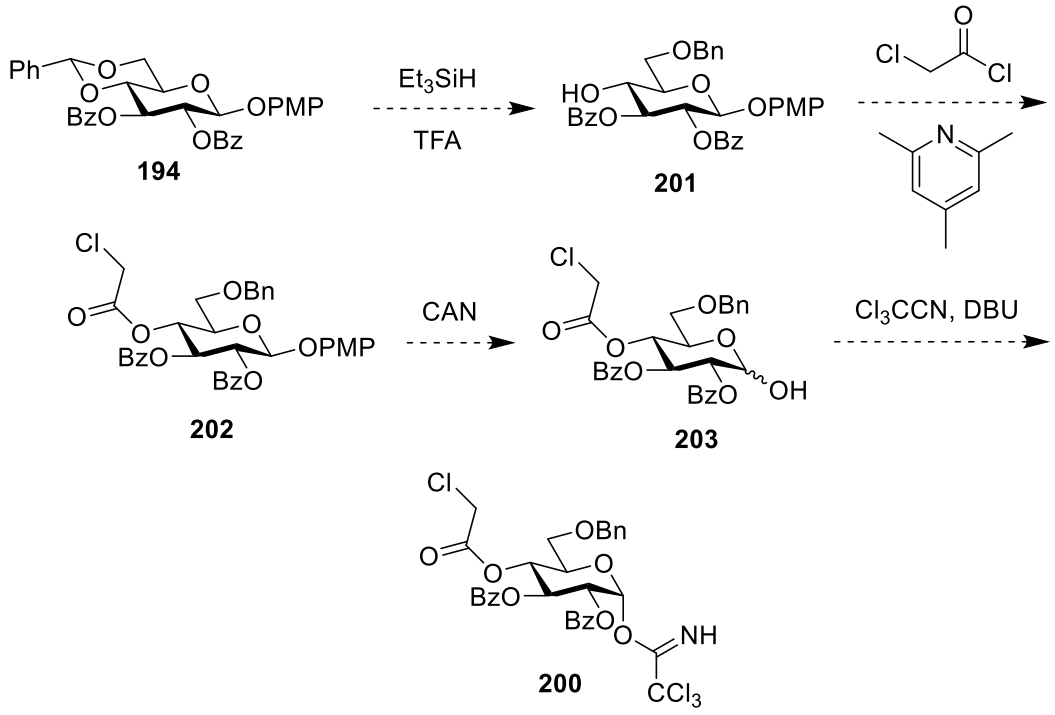
ether protecting group may be used to synthesise glycosyl acceptor **199** in 74 % yield over two steps using a flow-to-pot deprotection strategy; achieved by flowing glycosylation reaction solution into a “deprotection” solution to begin deprotection immediately following glycosylation. This approach circumvents the need for intermediary purification steps and increases reaction yield. Hence, the use of donor **191** appears to be an excellent basis for the preparation of β-1,6-glucans in future work, using acceptor **199** as the foundation for preparing a library of β-1,6-glucan oligosaccharides.

The I-Tag supported flow glycosylation strategy requires further refinement to become more pragmatic and widely applicable. However, the successful development of the method thus far suggests it may constitute a useful contribution to glycoscience in the future. There are several areas where further work would propel the utility of the method and our understanding of it.

Firstly, silyl ether protected donors were discovered to undergo unexpected deprotection during flow glycosylations. Further exploration of the subtle reactivity differences between different flow and batch reactors would make for an interesting study to shed light on this phenomenon. One could explore reactions using various glycosyl donors bearing silyl ether protecting groups with different Lewis acid glycosylation promoters. Reactions could be performed in batch and flow reactors of different sizes; altering stirring rates, residence times, solvent and concentration to determine how they affect the propensity for silyl ether deprotection and formation of 1,6-anhydro sugars. This would help to build up a practical picture of how reactivity fluctuates dependent on these parameters.

The methodology should also be explored using a diverse range of glycosyl donors, with carbohydrate structures that feature in biologically relevant oligosaccharides. For instance, galactose and mannose derived donors could be tested, as well as 2-amino-2-deoxy sugars. Glycosyl donor **200**, which should theoretically be easily accessible from previously synthesised compounds, could be used to construct β-1,4-glucans. A suggested synthesis of **200** is shown in **Scheme 82**. Beginning from previously prepared benzylidene **194**, regioselective reductive ring opening would furnish secondary alcohol **201**. Subsequent treatment with chloroacetyl chloride and 2,4,6-collidine would install the chloroacetyl group

at C-4 to give **202**. Anomeric *para*-methoxyphenyl group removal and introduction of the trichloroacetimidate group would furnish donor **200**.



Scheme 82. *Potential synthetic pathway to donor **200** that might be used for construction of β-1,4-glucans.*

Whilst the chloroacetyl protecting group installed at the C-6 position on donor **167** caused neighbouring group migration issues during deprotection, that ought not to occur in the case of donor **200**. In the publication by Vliegenthart and co-workers[162] describing amine DABCO as a selective dechloroacetylation reagent, the researchers used substrates that were structurally similar to **200** (**Scheme 67**). This suggests that the C-4 chloroacetyl protecting group on a glucose derived monosaccharide can be quickly and easily removed without the C-3 benzoyl group migrating to C-4. Thus, donor **200** should work well as the basis for I-Tag supported construction of β-1,4-glucans in flow with rapid chloroacetate deprotection under basic conditions.

Another area where future work might improve the usefulness of this glycosylation strategy is in the synthesis of 1,2-*cis* glycosides. Thus far, only 1,2-*trans* glycosides (or anomeric mixtures) have been synthesised owing to the difficulties in controlling the

stereochemistry of the glycosidic bond. However, one of the approaches discussed in section **2.2.** for 1,2-*cis* glycoside preparation could be incorporated into this I-Tag supported flow strategy, allowing a wider array of carbohydrate architectures to be accessed.

Investigation of some of the suggested future research directions and the resultant preparation of diverse oligosaccharides would demonstrate the utility and versatility of I-Tag supported glycosylation in flow. This should form a convincing argument for adoption of this method amongst glycoscientists to expedite the preparation of carbohydrate target molecules.

6. Experimental

6.1. General Experimental Details

Chemicals were purchased and used without further purification, except for benzaldehyde dimethyl acetal and trimethylsilyl trifluoromethanesulfonate, which were distilled prior to use. Dry solvents were obtained by distillation using standard procedures or by passage through a column of anhydrous alumina using equipment from Anhydrous Engineering (University of Bristol) based on the Grubbs' design.[165] Reactions requiring anhydrous conditions were performed under nitrogen; glassware was either flame dried immediately prior to use or placed in an oven (155 °C) for at least 3 hours and allowed to cool under reduced pressure; liquid reagents, solutions or solvents were added via syringe through rubber septa; solid reagents were added via Schlenk type adapters. Reactions were monitored by TLC on Kieselgel 60 F_{254} (Merck). Detection was by examination under UV light (254 nm) and by charring with 10 % sulfuric acid in ethanol or potassium permanganate solution. Flash column chromatography was performed using silica gel [Merck, 230−400 mesh (40−63 μm)]. The crude material was applied to the column as a solution or by pre-adsorption on silica, as appropriate. Extracts were concentrated *in vacuo* using both a Büchi rotary evaporator (bath temperatures up to 60 °C) at a pressure of either 15 mmHg (diaphragm pump) or 0.1 mmHg (oil pump), as appropriate, and a high vacuum line at room temperature. Water soluble compounds were freeze dried on a Lyotrap Plus (LTE Scientific LTD).

^{1}H, ^{13}C and ^{19}F NMR spectra were measured at 25 ˚C in the solvent stated with Varian, Bruker or Jeol spectrometers operating at the field strengths listed. Chemical shifts are quoted in parts per million to two decimal places with spectra referenced to the residual solvent peak ($CDCl_3$: ^{1}H = 7.26 ppm and ^{13}C = 77.16 ppm; CD_3CN: ^{1}H = 1.94 ppm and ^{13}C = 1.32 and 118.26 ppm; CD_3OD: ^{1}H = 3.31 ppm and ^{13}C = 49.00 ppm; D_2O: ^{1}H = 4.79 ppm), ^{19}F NMR chemical shifts are also quoted in ppm but are unreferenced, coupling constants (*J*) given in Hertz (Hz) as observed. Multiplicities are abbreviated as: app (apparent), b (broad), s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet) or combinations thereof. The abbreviation ax denotes an axial proton, whilst eq denotes an equatorial proton. Assignments

of ^{1}H and ^{13}C NMR signals were made where possible, using COSY, TOCSY, HSQC and HMBC experiments. Assignments for carbohydrate derivatives are shown in **Figure 30**:

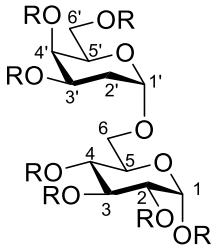


Figure 30. *Format for NMR assignments of carbohydrate chemicals synthesised.*

High- and low-resolution mass spectra were obtained from the University of Bristol Mass Spectrometry Service by ESI or MALDI modes. Reactions followed by MALDI were analysed using a Bruker Daltonics UltrafleXtreme time-of-flight/time-of-flight mass spectrometer. 2,5-Dihydroxybenzoic acid in MeCN was used as matrix. LC-MS spectra were recorded on a LC Packings Famos system with a Bruker esquire 6000 mass spectrometer. Infrared spectra were recorded in the range 4000 – 400 cm^{-1} on a Perkin Elmer Spectrum Two Spectrometer. Optical rotations were measured on a Bellingham + Stanley ADP220 polarimeter. The units of the specific rotation, (deg·mL)/(g·dm), are implicit and are not included with the reported value. Concentration c is given in g/100 mL. Preparative HPLC was performed on a Grace Discovery Sciences Reveleris Prep System. For purification, the instrument was set to monitor the ELSD signal in addition to UV detection at 220 nm, 254 nm and 280 nm. For reverse phase purifications a Phenomenex Luna 5 µm C18(2) 100 Å AXIA packed (250 x 21.2 mm) column was used. For normal phase purifications, FlashPure EcoFlex cartridges loaded with either 4 g, 12 g, 40 g or 120 g of silica were used.

Named, but unnumbered compounds in the experimental section are listed first, followed by numbered compounds in numerical order. The final compounds contained in this experimental are: 1,2,3,4,6-penta-*O*-acetyl-D-galactopyranoside, 1,2,3,4,6-penta-*O*-acetyl-D-glucopyranoside, **41**, **44**, **53**, **54**, **62**, **74**, **92**, **97**, **100**, **102**, **120**, **121**, **122**, **123**, **125**, **126**, **130**, **131**, **139**, **140**, **147**, **151** (two alternate routes to this compound), **152**, **155**, **156**, **157**, **159**, **160**, **161**, **163**, **166**, **167**, **168**, **170**, **172**, **174**, **177**, **178**, **179**, **181**/**183**/**184**/**185**, **186**, **187**, **190**, **191**, **192**, **194**, **199**, **204**, **205**, **206**, **207**, **208**, **209**, **210**, **211**, **212**, **213**, **214**, **215**, **216**, **217**, **218**,

219, **220**, **221**, **222** and **223**. Compounds that are not explicitly numbered in the main text of this thesis are instead numbered for the first time in the experimental. Compounds **204**-**208** pertain to section **4.1.1. Glycosyl Acceptor Syntheses**, being either precursor compounds or glycosyl acceptors. Compounds **209**-**217** apply to section **4.3.2. Acceptor Scope**, being glycosylation products from the palladium catalysed glycosylation method described in that section. Compounds **218**-**220** are relevant to section **4.6.3. Reactions Using Triisopropylsilyl Ether Protected Donors**, being precursor compounds to the trichloroacetimidate donors reported in that section. Finally, compounds **221**-**223** pertain to section **4.6.2. Exploration of Substrate Scope**, being glycosylation products from the I-Tag supported flow glycosylation reactions described in **Table 13**.

6.2. Synthetic Procedures

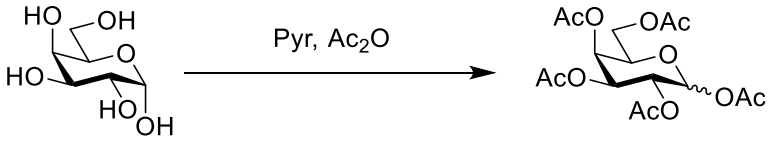
General Procedure for Palladium Catalysed Glycosylation Reactions

Glycal glycosyl donor, glycosyl acceptor, metal catalyst and ligand were weighed into an oven-dried microwave vial and the vial was sealed with a cap with septum. The reagents were dried under vacuum for at least 30 min, after which time anhydrous DCM was added under a nitrogen atmosphere. The vial cap was wrapped with Parafilm and the reaction mixture left to stir (500 rpm) at 50 ℃ under a nitrogen atmosphere. Upon complete reaction as judged by TLC and/or ^{1}H NMR spectroscopy, the reaction was quenched by passing the mixture through a pad of Celite, with additional washing with DCM. Solvent was then removed under reduced pressure and the crude product was purified by chromatography.

General Procedure for I-Tag Glycosylations in Flow

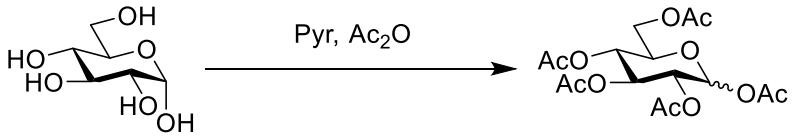
Glycosyl acceptor and glycosyl donor were placed in a dry vial and dried under vacuum for 30 min. Another dry vial was also placed under vacuum for 30 min. After this drying period, anhydrous solvent was added to the donor/acceptor vial under nitrogen. To the other vial, anhydrous solvent was added under nitrogen, followed by trimethylsilyl trifluoromethanesulfonate. The flow microreactor and attached tubing were flushed with nitrogen. The donor/acceptor solution and TMSOTf solution were each taken up in a syringe and installed onto a syringe pump. The solutions were then injected into the microreactor (total internal volume of reactor chip and outlet tubing = 32.8 µL) at the desired flow rate corresponding to the residence time (15 seconds, 65.60 µL/min in each syringe for a combined flow rate of 131.20 µL/min in reactor zone) via the inlet tubing. The mixture that flowed from the microreactor was dropped in a flask containing reagent grade solvent in air to quench the reaction. Reaction solution was collected for a specific time, after which time the reaction mixture solvent was removed under reduced pressure, unless otherwise stated. The crude product was dissolved in DCM and washed with water, then the water was extracted with further portions of DCM. The DCM phases were collected, dried with magnesium sulfate, filtered and the solvent was removed under reduced pressure. The dried residue was washed with appropriate solvents with sonication then dried under reduced pressure to yield the title compound.

1,2,3,4,6-Penta-*O*-acetyl-D-galactopyranoside



α-D-galactopyranose (3.00 g, 16.65 mmol) was dissolved in pyridine (48 mL) and acetic anhydride (24 mL) was added to the resulting solution. The mixture was stirred at RT for 60 h. The mixture was then diluted with DCM (30 mL) and washed with 1 M HCl $_{(aq.)}$ (3 x 30 mL), 5 M HCl $_{(aq.)}$ (30 mL) and brine (30 mL). The organic phase was dried using magnesium sulfate and filtered. Solvent was removed under reduced pressure to yield the crude product as a light brown oil that slowly crystallised to an off-white solid. The crude product was then recrystallised from ethanol, the solid product formed was vacuum filtered using Hirsch apparatus and dried under vacuum for 3 h to yield a mixture of anomers (α:β = 11:1) of the title compound as an off-white solid (2.53 g, 39 %), with spectroscopic details in accordance with the literature;[166] **^{1}H NMR** δ_H (400 MHz, Chloroform-*d*) 6.37 (1 H, d, *J* 1.8, H-1α), 5.69 (1 H, d, *J* 8.3, H-1β), 5.51 – 5.47 (1 H, m, H-4α), 5.37 – 5.28 (2 H, m, H-2α, H-3α), 5.07 (1 H, dd, *J* 10.4, 3.2, H-3β), 4.33 (1 H, td, *J* 6.7, 1.4, H-5α), 4.16 – 4.01 (2 H, m, H-6aα, H-6bα), 2.17 – 2.06 (6 H, m, C(O)CH_3α), 2.03 (3 H, s, C(O)CH_3α), 2.01 (3 H, s, C(O)CH_3α), 1.99 (3 H, s, C(O)CH_3α); **^{13}C NMR** δ_C (101 MHz, Chloroform-*d*) δ_C 170.49, 170.26, 170.00, 169.05 (5 C=O), 89.83 (C-1α), 68.88 (C-5α), 67.54, 67.48 (C-3α and C-4α), 66.56 (C-2α), 61.37 (C-6α), 21.01, 20.78, 20.77, 20.73, 20.67 (5 C(O)*C*H_3); **m/z** (ESI-MS+) $C_{16}H_{22}O_{11}Na^+$ ([M + Na]$^+$) calculated: 413.1; found 413.1.

1,2,3,4,6-Penta-*O*-acetyl-D-glucopyranoside

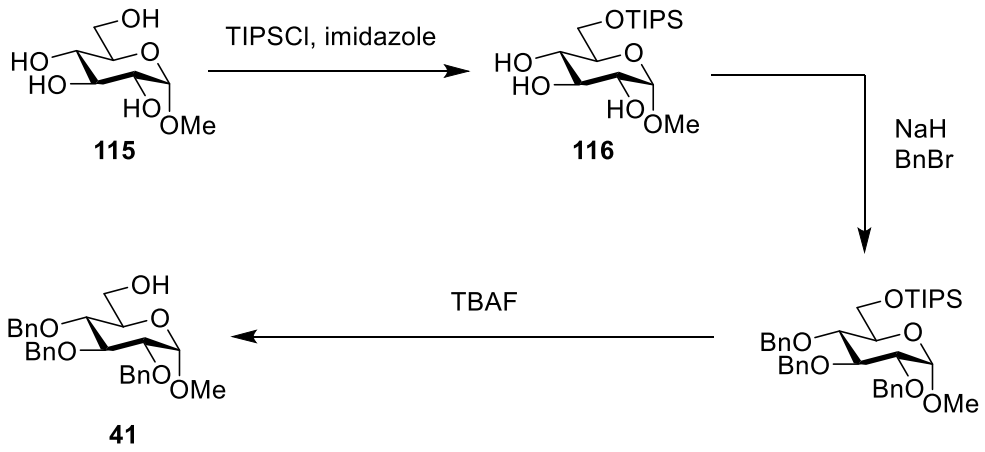


1

α-D-glucopyranose (7.50 g, 41.63 mmol) was dissolved in pyridine (120 mL) and acetic anhydride (60 mL) was added to the resulting solution. The mixture was stirred at RT for 21 h.

The mixture was then diluted with DCM (75 mL) and washed with 1 M $HCl_{(aq.)}$ (3 x 75 mL), 5 M $HCl_{(aq.)}$ (75 mL) and brine (75 mL). The organic phase was dried using magnesium sulfate and filtered. Solvent was removed under reduced pressure to yield a white solid that was dried under vacuum for 20 h to yield a mixture of anomers (α:β = 10:1) of the title compound as a white solid (13.85 g, 85 %), with spectroscopic details in accordance with the literature;[167] **^{1}H NMR** δ_H (400 MHz, Chloroform-*d*) 6.32 (1 H, d, *J* 3.6, H-1α), 5.70 (1 H, d, *J* 8.3, H-1β), 5.46 (1 H, t, *J* 9.9, H-3α), 5.23 (1 H, d, *J* 9.4, H-4β), 5.18 – 5.04 (2 H, m, H-2α, H-4α), 4.26 (1 H, dd, *J* 12.7, 4.2, H-6aα), 4.15 – 4.04 (2 H, m, H-5α, H-6bα), 2.17 (3 H, s, C(O)CH_3α), 2.08 (3 H, s, C(O)CH_3α), 2.03 (3 H, s, C(O)CH_3α), 2.01 (3 H, s, C(O)CH_3α), 2.00 (3 H, s, C(O)CH_3α); **^{13}C NMR** δ_C (101 MHz, Chloroform-*d*) 170.75, 170.34, 169.77, 169.51, 168.87 (5 C=O), 89.18 (C-1α), 69.94 (C-3α, C-5α), 69.30 (C-2α), 68.00 (C-4α), 61.57 (C-6α), 21.00, 20.82, 20.78, 20.68, 20.57 (5 C(O)*C*H_3); **m/z** (ESI-MS+) $C_{16}H_{22}O_{11}Na^+$ ([M + Na]$^+$) calculated: 413.11; found 413.10.

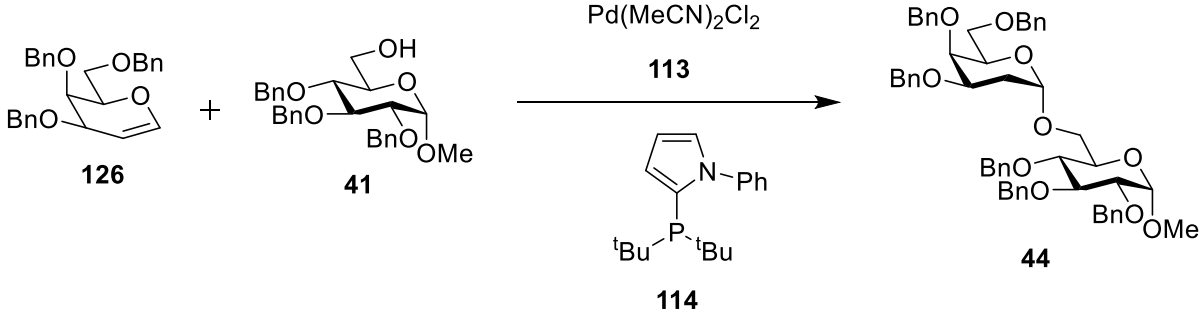
Methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside 41



Methyl α-D-glucopyranoside **115** (10.00 g, 51.50 mmol) was dried under vacuum for 1 h in a flame-dried flask before being dissolved in anhydrous DMF (200 mL) under a nitrogen atmosphere. Imidazole (8.77 g, 128.75 mmol) was added and the solution cooled to 0 °C. TIPSCl (11.25 mL, 52.53 mmol) was added dropwise and the resulting mixture was stirred at RT overnight. The solvent was then removed under reduced pressure and the residue was dissolved in DCM (300 mL). The resulting solution was washed with water (300 mL) and brine (300 mL), dried using magnesium sulfate and filtered. Removal of solvent under reduced pressure afforded silylated intermediate **116**. This intermediate was dissolved in anhydrous

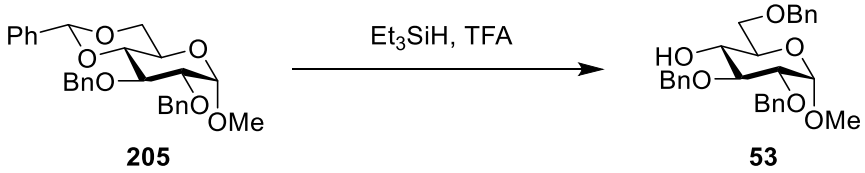
DMF (200 mL) in a flame-dried flask under a nitrogen atmosphere and cooled to 0 °C. Sodium hydride (60 % in mineral oil, 12.36 g, 309.00 mmol) was added and the mixture was stirred for 60 min at RT, after which time the mixture was again cooled to 0 °C. Benzyl bromide (36.70 mL, 309.00 mmol) was added dropwise and the reaction mixture was stirred for 18 h at RT, after which time methanol (50 mL) was added. Solvent was removed under reduced pressure and the residue was dissolved in DCM (300 mL). The resulting solution was washed with water (2 x 200 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure to afford a benzylated intermediate. This intermediate was partially purified by column chromatography (Hexane:EtOAc 19:1 → 16:1) then dissolved in a 1 M solution of TBAF in THF (61.80 mL, 61.80 mmol). The resulting solution was stirred for 2 h 15 min at RT. After this time the solvent was removed under reduced pressure and the residue was dissolved in DCM (300 mL), washed with water (2 x 200 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure to afford a crude product. The crude product was purified by column chromatography (Hexane:EtOAc 9:1 → 8:2 → 7:3 → 6:4) to afford the title compound **41** (14.80 g, 62 %) as a white solid, with spectroscopic details in accordance with the literature;[108] **^{1}H NMR** δ_H (400 MHz, Chloroform-*d*) 7.41 – 7.27 (15 H, m, H_{arom}), 4.99 (1 H, d, *J* 10.9, PhC*H*H), 4.93 – 4.76 (3 H, m, PhCH_2), 4.67 (1 H, d, *J* 9.1, PhC*H*H), 4.64 (1 H, d, *J* 8.1, PhCH*H*), 4.58 (1 H, d, *J* 3.6, H-1), 4.01 (1 H, t, *J* 9.3, H-3), 3.82 – 3.62 (3 H, m, H-5, H-6a, H-6b), 3.57 – 3.47 (2 H, m, H-2, H-4), 3.37 (3 H, s, OCH_3), 1.64 (1 H, br s, OH); **^{13}C NMR** δ_C (101 MHz, Chloroform-*d*) 138.87, 138.27, 138.25 (3 4° C_{arom}), 128.61, 128.54, 128.26, 128.17, 128.10, 128.08, 128.01, 127.75 (15 C_{arom}), 98.32 (C-1), 82.09 (C-3), 80.12 (C-2), 77.54 (C-4), 75.88, 75.16, 73.57 (3 PhCH_2), 70.79 (C-5), 62.01 (C-6), 55.32 (OCH_3); **m/z** (ESI-MS+) $C_{28}H_{32}O_6Na^+$ ([M + Na]$^+$) calculated: 487.2; found 487.2.

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(3,4,6-tri-*O*-benzyl-α-D-lyxo-hexapyranosyl)-α-D-glucopyranoside 44



Following the general procedure for palladium catalysed glycosylation reactions, glycosyl donor tri-*O*-benzyl-D-galactal **126** (0.050 g, 0.120 mmol), glycosyl acceptor methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **41** (0.042 g, 0.090 mmol), metal catalyst bis(acetonitrile)dichloropalladium (II) **113** (0.009 g, 0.036 mmol) and ligand 2-(di-*tert*-butylphosphanyl)-1-phenyl-1H-pyrrole **114** (0.010 g, 0.036 mmol) were dissolved in 2 mL of anhydrous DCM. The reaction mixture was stirred for 21 h before being quenched. Following purification by column chromatography (Hexane:EtOAc 8:1 → 5:1 → 4:1) the title compound **44** was obtained as a yellow oil (0.068 g, 86 %, α:β >30:1), with spectroscopic details in accordance with the literature;[108] **^{1}H NMR** δ_H (301 MHz, Chloroform-*d*) 7.42 – 7.17 (30 H, m, H_{arom}), 5.02 (1 H, app d, *J* 3.1, H-1'), 4.98 (1 H, d, *J* 10.8, PhC*H*H), 4.91 (1 H, d, *J* 11.6, PhC*H*H), 4.84 (1 H, d, *J* 10.9, PhC*H*H), 4.80 (1 H, d, *J* 11.0, PhCH*H*), 4.78 (1 H, d, *J* 12.2, PhC*H*H), 4.67 (1 H, d, *J* 12.2, PhCH*H*), 4.60 (1 H, d, *J* 3.7, H-1), 4.59 (1 H, d, *J* 11.6, PhCH*H*), 4.57 (2 H, s, PhCH_2), 4.52 (1 H, d, *J* 10.9, PhCH*H*), 4.40 (1 H, d, *J* 11.8, PhC*H*H), 4.33 (1 H, d, *J* 11.9, PhCH*H*), 3.98 (1 H, t, *J* 9.2, H-3), 3.90 – 3.83 (3 H, m, H-3', H-4', H-5'), 3.81 (1 H, dd, *J* 11.2, 4.6, H-6a), 3.71 (1 H, ddd, *J* 10.0, 4.6, 1.8, H-5), 3.61 (1 H, dd, *J* 11.4, 1.9, H-6b), 3.57 – 3.49 (2 H, m, H-6a', H-6b'), 3.51 (1 H, dd, *J* 9.7, 3.5, H-2) 3.46 (1 H, dd, *J* 9.9, 9.2, H-4), 3.31 (3 H, s, OCH_3), 2.20 (1 H, td, *J* 12.5, 3.7, H-2ax'), 2.01 (1 H, app dd, *J* 12.8, 4.5, H-2eq'); **^{13}C NMR** δ_C (76 MHz, Chloroform-*d*) 139.02, 138.88, 138.52, 138.39, 138.30, 138.28 (6 4° C_{arom}), 128.61, 128.55, 128.45, 128.34, 128.33, 128.19, 128.17, 128.05, 127.83, 127.82, 127.79, 127.72, 127.62, 127.54 (30 C_{arom}), 98.42 (C-1'), 98.00 (C-1), 82.26 (C-3), 80.15 (C-2), 78.05 (C-4), 75.95 (Ph*C*H_2), 75.10 (Ph*C*H_2), 74.42, 74.35 (C-3', Ph*C*H_2), 73.47 (Ph*C*H_2), 73.42 (Ph*C*H_2), 73.05 (C-4'), 70.37 (Ph*C*H_2), 70.21 (C-5'), 69.96 (C-5), 69.53 (C-6'), 66.16 (C-6), 55.16 (OCH_3) 31.16 (C-2'); **m/z** (ESI-MS+) $C_{55}H_{60}O_{10}Na^+$ ([M + Na]$^+$) calculated 903.41; found 903.41.

Methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside 53

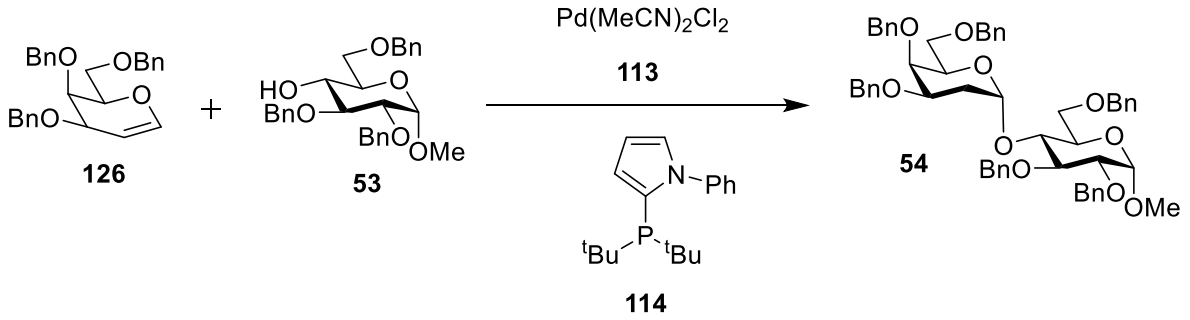


205 **53**

Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside **205** (8.50 g, 18.38 mmol) was dried under vacuum for 1 h in a flame-dried flask before being dissolved in

anhydrous DMF (180 mL) under a nitrogen atmosphere. The solution was cooled to 0 °C and triethylsilane (28.6 mL, 179.06 mmol) was added. Trifluoroacetic acid (14.1 mL, 184.26 mmol) was then added dropwise and the reaction mixture was left to stir at 0 °C for 30 min. When the reaction was judged to be complete by ^{1}H NMR spectroscopy, the reaction mixture was quenched by addition of triethylamine (26.4 mL) and methanol (22 mL). DCM (260 mL) was added to the reaction mixture and the organic phase washed with water (2 x 220 mL) and brine (220 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure to give a crude product as a yellow oil. Following purification by column chromatography (DCM:Et_2O 95:5) the title compound **53** was obtained as a pale yellow oil (3.16 g, 37 %) with spectroscopic details in accordance with the literature;[108] **^{1}H NMR** δ_H (400 MHz, Chloroform-*d*) 7.42 – 7.27 (15 H, m, H_{arom}), 5.01 (1 H, d, *J* 11.4, PhC*H*$_2$), 4.77 (1 H, d, *J* 12.2, PhC*H*$_2$), 4.74 (1 H, d, *J* 11.5, PhC*H*$_2$), 4.66 (1 H, d, *J* 12.0, PhC*H*$_2$), 4.64 (1 H, d, *J* 3.5, H-1), 4.60 (1 H, d, *J* 12.2, PhC*H*$_2$), 4.54 (1 H, d, *J* 12.1, PhC*H*$_2$), 3.79 (1 H, t, *J* 9.2, H-3), 3.75 – 3.65 (3 H, m, H-5, H-6a, H-6b), 3.61 (1 H, t, *J* 9.1, H-4), 3.54 (1 H, dd, *J* 9.5, 3.5, H-2), 3.39 (3 H, s, OCH_3), 2.33 (1 H, s, OH); **^{13}C NMR** δ_C (101 MHz, Chloroform-*d*) 138.93, 138.18, 138.13 (3 4° C_{arom}), 128.72, 128.60, 128.49, 128.26, 128.13, 128.08, 127.98, 127.77, 127.75 (15 C_{arom}), 98.33 (C-1), 81.59 (C-3), 79.72 (C-2), 75.57 (Ph*C*H$_2$), 73.72 (Ph*C*H$_2$), 73.30 (Ph*C*H$_2$), 70.86 (C-4), 70.01 (C-5), 69.61 (C-6), 55.39 (OCH_3); **m/z** (ESI-MS+) $C_{28}H_{32}O_6Na^+$ ([M + Na]$^+$) calculated: 487.21; found 487.21.

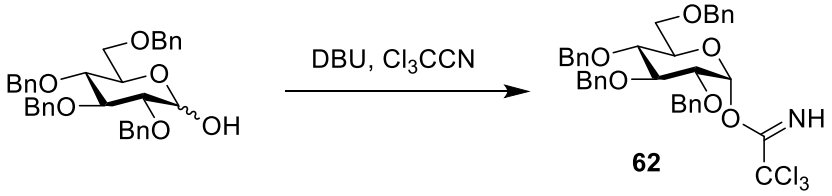
Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-benzyl-α-D-lyxo-hexapyranosyl)-α-D-glucopyranoside 54



Following the general procedure for palladium catalysed glycosylation reactions, glycosyl donor tri-*O*-benzyl-D-galactal **126** (0.100 g, 0.240 mmol), glycosyl acceptor methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside **53** (0.084 g, 0.180 mmol), metal catalyst

bis(acetonitrile)dichloropalladium (II) **113** (0.019 g, 0.072 mmol) and ligand 2-(di-*tert*-butylphosphanyl)-1-phenyl-1H-pyrrole **114** (0.021 g, 0.072 mmol) were dissolved in 2 mL of anhydrous DCM. The reaction mixture was stirred for 45 h before being quenched. Following purification by column chromatography (Hexane:EtOAc 9:2) the title compound **54** was obtained as a yellow oil (0.114 g, 72 %, α:β = 4:1), with spectroscopic details in accordance with the literature.[108] Note that only characteristic, non-overlapping, distinguishable NMR spectroscopy signals are given for the minor β anomer; **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) **α anomer:** 7.38 – 7.17 (30 H, m, H_{arom}), 5.47 (1 H, app d, *J* 3.7, H-1'), 4.99 (1 H, d, *J* 10.8, PhC*H*H), 4.88 (1 H, d, *J* 11.7, PhC*H*H), 4.73 (1 H, d, *J* 12.1, PhC*H*H), 4.65 (1 H, d, *J* 11.0, PhCH*H*), 4.64 – 4.55 (3 H, m, H-1, PhCH_2), 4.57 – 4.48 (3 H, m, PhCH_2), 4.38 (1 H, d, *J* 12.0, PhCH*H*), 4.36 (1 H, d, *J* 11.7, PhC*H*H), 4.30 (1 H, d, *J* 11.7, PhCH*H*), 3.87 (1 H, t, *J* 9.1, H-3), 3.85 (1 H, bs, H-4'), 3.82 (1 H, t, *J* 6.6, H-5'), 3.78 (1 H, ddd, *J* 12.2, 4.5, 2.3, H-3'), 3.74 – 3.68 (1 H, m, H-5), 3.68 – 3.59 (3 H, m, H-4, H-6a, H-6b), 3.51 (1 H, dd, *J* 9.8, 3.4, H-2), 3.51 – 3.43 (2 H, m, H-6a', H-6b'), 3.39 (3 H, s, OCH_3), 2.13 (1 H, td, *J* 12.4, 4.0, H-2ax',), 1.87 (1 H, dd, *J* 12.5, 4.5, H-2eq'); **β anomer:** 5.24 (1H, app d, *J* = 3.5 Hz, H-1'), 2.24 (1H, td, *J* = 12,4, 3.7 Hz, H-2ax'); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) **α anomer:** 138.91, 138.60, 138.58, 138.53, 138.20, 138.16 (6 4° C_{arom}), 128.58, 128.53, 128.46, 128.44, 128.33, 128.30, 128.26, 128.05, 127.95, 127.78, 127.76, 127.73, 127.68, 127.67, 127.63, 127.44, 127.43 (30 C_{arom}), 99.82 (C-1'), 97.92 (C-1), 82.20 (C-3), 80.17 (C-2), 76.04 (C-4), 75.66 (Ph*C*H_2), 74.62 (C-3'), 74.38 (Ph*C*H_2), 73.59 (Ph*C*H_2), 73.41 (Ph*C*H_2), 73.19 (Ph*C*H_2), 72.89 (C-4'), 70.85 (C-5'), 70.53 (Ph*C*H_2), 70.04 (C-5), 69.73, 69.69 (C-6, C-6'), 55.34 (OCH_3), 31.76 (C-2'); **β anomer:** 93.5 (C-1'); **m/z** (ESI-MS+) $C_{55}H_{60}O_{10}Na^+$ ([M + Na]$^+$) calculated 903.4; found 903.4.

2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl trichloroacetimidate 62

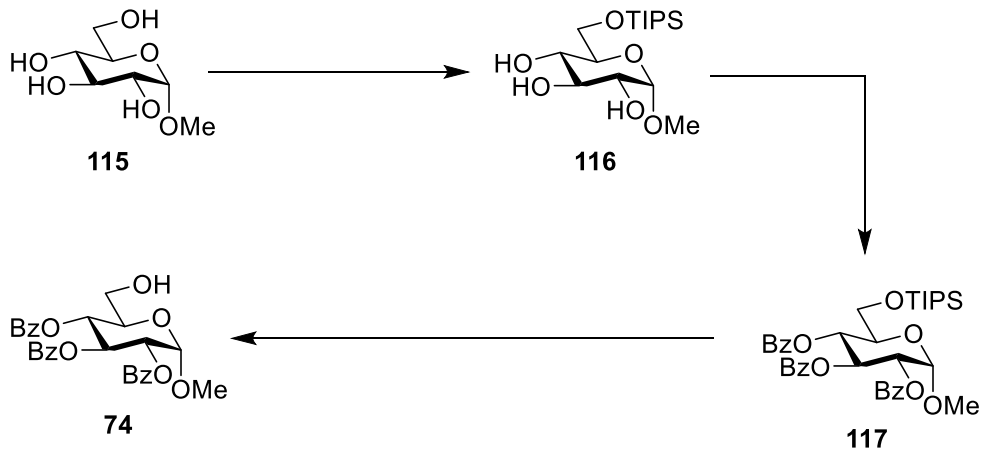


62

2,3,4,6-Tetra-*O*-benzyl-D-glucopyranose (4.00 g, 7.40 mmol) was dried under vacuum for 1 h before being dissolved in anhydrous DCM (45 mL). Trichloroacetonitrile (11.13 mL, 111.00 mmol) and DBU (0.55 mL, 3.70 mmol) were added and the resulting solution was

stirred at RT for 55 min under a nitrogen atmosphere, after which time TLC (Hexane:EtOAc 8:2) showed the reaction to be complete. Solvent was then removed under reduced pressure and the crude product was purified by column chromatography (Hexane:EtOAc 9:1) using neutralised silica (1 % NEt_3) to give the title compound **62** as a colourless syrup (4.88 g, 96 %) with spectroscopic details in accordance with the literature;[168] **^{1}H NMR** δ_H (400 MHz, Chloroform-*d*) 8.59 (1 H, s NH), 7.37 – 7.27 (18 H, m, H_{arom}), 7.19 – 7.12 (2 H, m, H_{arom}), 6.54 (1 H, d, *J* 3.4, H-1), 4.97 (1 H, dd, *J* 11.1, 1.7, PhC*H*H), 4.90 – 4.81 (2 H, m, PhCH_2), 4.76 (1 H, d, *J* 11.7, PhC*H*H), 4.72 – 4.66 (1 H, m, PhCH*H*), 4.62 (1 H, d, *J* 12.1, PhC*H*H), 4.54 (1 H, d, *J* 10.7, PhCH*H*), 4.48 (1 H, d, *J* 12.0, PhCH*H*), 4.06 (1 H, td, *J* 9.4, 1.7, H-3), 4.00 (1 H, dd, *J* 10.2, 2.5, H-5), 3.83 – 3.76 (3 H, m, H-2, H-4, H-6a), 3.68 (1 H, dd, *J* 11.0, 1.9, H-6b); **^{13}C NMR** δ_C (101 MHz, Chloroform-*d*) 161.45 (CNH), 138.73, 138.17, 138.08, 137.96 (4 4° C_{arom}), 128.54, 128.52, 128.49, 128.48, 128.20, 128.13, 128.08, 127.95, 127.86, 127.84, 127.76, 127.74 (C_{arom}), 94.51 (C-1), 91.41 (CCl_3), 81.51 (C-3), 79.49 (C-2), 76.94 (C-4), 75.77 (Ph*C*H_2), 75.46 (Ph*C*H_2), 73.61 (Ph*C*H_2), 73.25 (C-5), 73.01 (Ph*C*H_2), 68.17 (C-6); **m/z** (ESI-MS+) $C_{36}H_{36}Cl_3NO_6Na^+$ ([M + Na]$^+$) calculated 706.15; found 706.15.

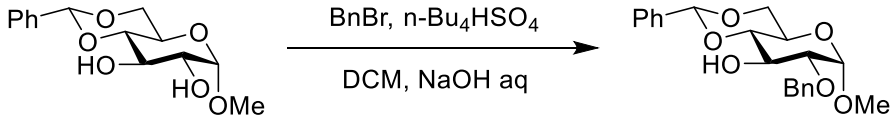
Methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside 74



Methyl α-D-glucopyranoside **115** (7.00 g, 36.05 mmol) was dried under vacuum for 1 h in a flame-dried flask before being dissolved in anhydrous pyridine (210 mL) under a nitrogen atmosphere. Imidazole (5.39 g, 79.17 mmol) was added and the solution was cooled to 0 ℃. TIPSCl (8.4 mL, 39.26 mmol) was then added dropwise and the resulting mixture was stirred at RT for 16 h under nitrogen, after which time benzoyl chloride (33.6 mL, 289.22 mmol) was

added and the reaction mixture was stirred for a further 24 h under nitrogen at RT. Methanol (14 mL) was then added and the reaction mixture was diluted with DCM (200 mL). The resulting solution was washed with 1 M HCl $_{(aq.)}$ (2 x 200 mL), $NaHCO_{3\ (sat.\ aq.)}$ (200 mL) and water (200 mL). The organic phase was dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure to afford fully protected intermediate **117**. Intermediate **117** was dissolved in a solution of THF (112 mL), water (42 mL) and TFA (30 mL) and then stirred for 48 h at RT, after which time the solvent was removed under reduced pressure by forming an azeotrope with toluene. The crude product was purified by column chromatography (Hexane:EtOAc 12:1 → 6:1 → 4:1 → 3:1) to afford the title compound **74** (12.32 g, 67 %) as a white solid, with spectroscopic details in accordance with the literature;[108] **^{1}H NMR** δ_H (400 MHz, Chloroform-*d*) 8.01 – 7.94 (4 H, m, H_{arom}), 7.91 – 7.85 (2 H, m, H_{arom}), 7.58 – 7.47 (2 H, m, H_{arom}), 7.46 – 7.34 (5 H, m, H_{arom}), 7.33 – 7.23 (2 H, m, H_{arom}), 6.24 (1 H, t, *J* 9.7, H-3), 5.49 (1 H, t, *J* 9.9, H-4), 5.33 – 5.23 (2 H, m, H-2, H-1), 4.05 (1 H, dt, *J* 10.2, 2.9, H-5), 3.86 (1 H, dd, *J* 13.0, 2.2, H-6a), 3.75 (1 H, dd, *J* 13.0, 3.7, H-6b), 3.59 (1 H, br s, OH), 3.47 (3 H, s, OCH_3); **^{13}C NMR** δ_C (101 MHz, Chloroform-*d*) 166.71, 166.00, 165.98 (3 C=O), 133.92, 133.54, 133.33 (3 4° C_{arom}), 130.15, 130.06, 129.79, 129.27, 129.13, 128.68, 128.57, 128.56, 128.45 (15 C_{arom}), 97.29 (C-1), 72.16 (C-2), 70.21 (C-3), 69.80 (C-5), 69.72 (C-4), 61.20 (C-6), 55.83 (OCH_3); **m/z** (ESI-MS+) $C_{28}H_{26}O_9Na^+$ ([M + Na]$^+$) calculated: 529.15; found 529.14; $C_{27}H_{23}O_8^+$ ([M – OCH_3]$^+$) calculated: 475.14; found 475.14.

Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside 92

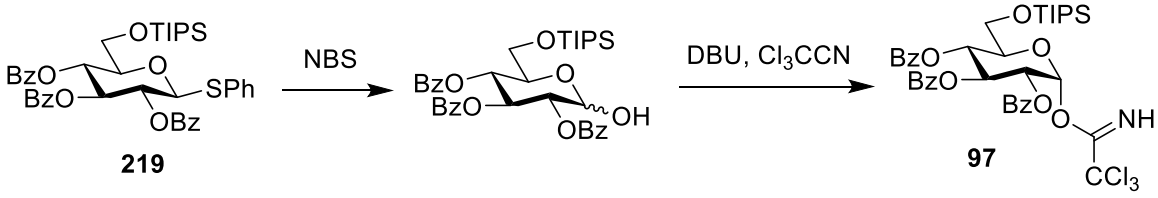


204 **92**

Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside **204** (6.50 g, 23.03 mmol) was dissolved in a mixture of DCM (200 mL), $NaOH_{\ aq.}$ (0.68 M, 50.8 mL), benzyl bromide (3.3 mL, 27.63 mmol) and tetrabutylammonium hydrogensulfate (1.56 g, 4.61 mmol). The mixture was stirred vigorously for 24 h at RT. The organic phase was then separated, washed with water (150 mL) and brine (150 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. Following purification by column chromatography

(Hexane:EtOAc, 8:2 → 3:1) the title compound **92** was obtained as a white solid (3.02 g, 35 %) with spectroscopic details in accordance with the literature;[108] **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 7.50 (2 H, dd, *J* 7.0, 2.4, H_{arom}), 7.46 – 7.28 (8 H, m, H_{arom}), 5.52 (1 H, s, PhC(*H*)OO), 4.79 (1 H, d, *J* 12.1, PhC*H*H), 4.70 (1 H, d, *J* 12.2, PhCH*H*), 4.62 (1 H, d, *J* 3.7, H-1), 4.26 (1 H, dd, *J* 10.1, 4.8, H-6a), 4.16 (1 H, td, *J* 9.3, 2.2, H-3), 3.82 (1 H, td, *J* 9.9, 4.8, H-5), 3.70 (1 H, t, *J* 10.3, H-6b), 3.54 – 3.42 (2 H, m, H-2, H-4), 3.38 (3 H, s, OCH_3), 2.72 (1 H, d, *J* 2.3, OH); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) 138.01, 137.18 (2 4° C_{arom}), 129.26, 128.65, 128.37, 128.23, 128.18, 126.42 (C_{arom}), 102.03 (Ph*C*(H)OO), 98.72 (C-1), 81.35 (C-4), 79.66 (C-2), 73.43 (Ph*C*H_2), 70.34 (C-3), 69.06 (C-6), 62.11 (C-5), 55.46 (OCH_3); **m/z** (ESI-MS+) $C_{21}H_{24}O_6Na^+$ ([M + Na]$^+$) calculated 395.1; found 395.1.

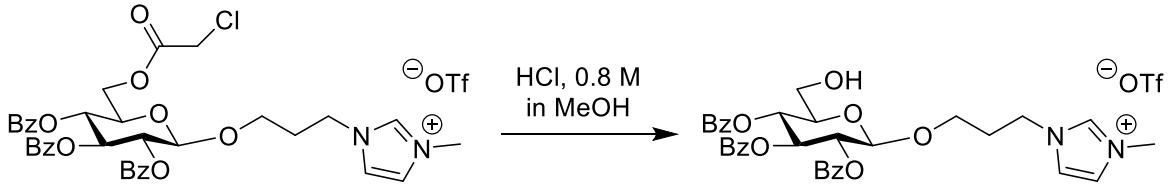
2,3,4-Tri-*O*-benzoyl-6-*O*-triisopropylsilyl-α-D-glucopyranosyl trichloroacetimidate 97



Phenyl 2,3,4-tri-*O*-benzoyl-6-*O*-triisopropylsilyl-β-D-thioglucopyranoside **219** (4.87 g, 6.57 mmol) was dissolved in a 10:1 acetone:water mixture (165 mL) and cooled to 0 °C. NBS (2.34 g, 13.14 mmol) that had been freshly recrystallised from water was added and the resulting solution was stirred for 100 min at RT after which time TLC (Hexane:EtOAc 8:2) showed the reaction to be complete. Most acetone solvent was removed under reduced pressure and DCM (200 mL) was added to the residue. The resulting organic solution was washed with $NaHCO_{3\ (sat.\ aq.)}$ (35 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (Hexane:EtOAc 95:5 → 92:8 → 87:13) to give the free hemiacetal intermediate as an anomeric mixture (2.74 g, 4.22 mmol). This intermediate was dried under vacuum for 1 h before being dissolved in anhydrous DCM (100 mL). Trichloroacetonitrile (6.35 mL, 63.30 mmol) and DBU (0.31 mL, 2.11 mmol) were added and the resulting solution was stirred at RT for 1 h under a nitrogen atmosphere, after which time TLC (Hexane:EtOAc 8:2) showed the reaction to be complete. Solvent was then removed under reduced pressure

and the crude product was purified by column chromatography (Hexane:EtOAc 94:6) using neutralised silica (1 % NEt_3) to give the title compound **97** as a white solid (2.79 g, 53 % over 2 steps) with spectroscopic details in accordance with the literature;[144] **^{1}H NMR** δ_H (400 MHz, Chloroform-*d*) 8.57 (1 H, s, NH), 7.95 (4 H, m, H_{arom}), 7.91 – 7.84 (2 H, m, H_{arom}), 7.55 – 7.26 (9 H, m, H_{arom}), 6.83 (1 H, d, *J* 3.6, H-1), 6.23 (1 H, t, *J* 10.0, H-3), 5.74 (1 H, t, *J* 10.0, H-4), 5.54 (1 H, dd, *J* 10.2, 3.6, H-2), 4.37 (1 H, dt, *J* 10.3, 3.6, H-5), 3.98 – 3.88 (2 H, m, H-6a, H-6b), 1.09 – 0.94 (21 H, m, TIPS); **^{13}C NMR** δ_C (101 MHz, Chloroform-*d*) 165.91, 165.61, 165.21 (3 C=O), 160.72 (CNH), 133.58, 133.41, 133.28, 130.06, 129.95, 129.89, 129.31, 129.24, 128.88, 128.53, 128.48, 128.44 (C_{arom}), 93.50 (C-1), 91.07 (CCl_3), 73.94 (C-5), 71.16 (C-2), 70.69 (C-3), 68.80 (C-4), 62.63 (C-6), 18.01, 12.06 (TIPS); **m/z** (ESI-MS+) $C_{38}H_{44}Cl_3NO_9SiNa^+$ ([M + Na]$^+$) calculated 814.2; found 814.2; $C_{36}H_{43}O_8Si^+$ ([M – OC(NH)CCl_3]$^+$) calculated 631.3; found 631.3.

3-(3-Methylimidazolium)-1-propyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside trifluoromethanesulfonate 100

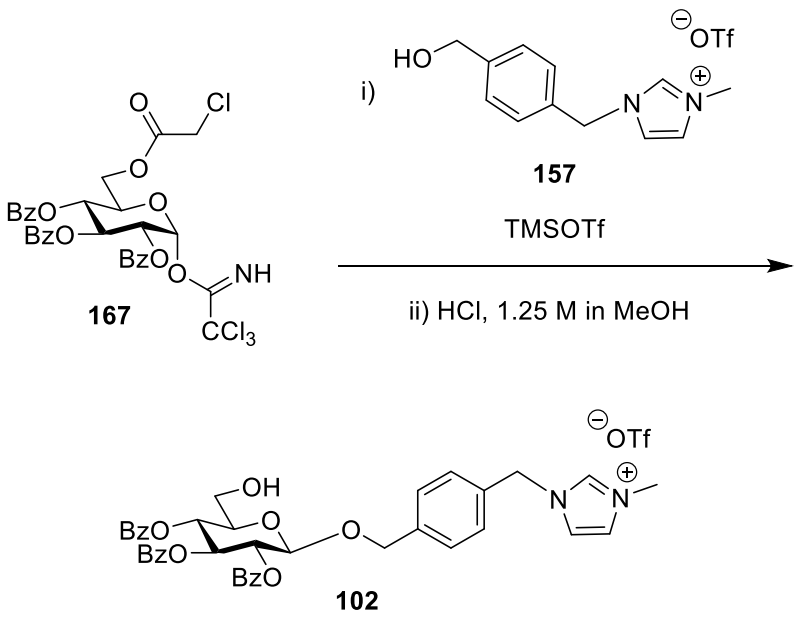


172 **100**

3-(3-Methylimidazolium)-1-propyl 2,3,4-tri-*O*-benzoyl-6-*O*-chloroacetyl-β-D-glucopyranoside trifluoromethanesulfonate **172** (0.0517 g, 0.061 mmol) was dissolved in a mixture of DCM (1 mL) and a solution of HCl, 0.8 M in MeOH (1.54 mL, 1.229 mmol). The resulting solution was stirred at RT for 16 h in air, after which time TLC showed the reaction to be complete. The reaction mixture was diluted with DCM (5 mL) and water (5 mL). Product was extracted into the DCM phase, then the aqueous phase was washed with further DCM portions (2 x 5 mL) and all DCM portions were combined, dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. The crude residue was washed with Et_2O (3 x 5 mL) then dried under reduced pressure to yield the title compound **100** as an off-white solid (0.0351 g, 75 %) with spectroscopic details in accordance with the literature;[144] **^{1}H NMR** δ_H (400 MHz, Acetonitrile-d_3) 8.45 (1 H, s, NCHN), 7.95 – 7.89 (4 H, m, H_{arom}), 7.79 –

7.74 (2 H, m, H_{arom}), 7.62 – 7.57 (2 H, m, H_{arom}), 7.52 (1 H, ddt, *J* 8.8, 7.2, 1.3, H_{arom}), 7.48 – 7.41 (4 H, m, H_{arom}), 7.38 – 7.33 (2 H, m, H_{arom}), 7.29 (2 H, app d, *J* 1.6, NC*H*CHN, NCHC*H*N), 5.87 (1 H, t, *J* 9.6, H-3), 5.53 (1 H, t, *J* 9.7, H-4), 5.37 (1 H, dd, *J* 9.8, 8.0, H-2), 4.96 (1 H, d, *J* 8.0, H-1), 4.24 – 4.10 (2 H, m, NCH_2), 3.96 (1 H, ddd, *J* 10.0, 5.0, 2.4, H-5), 3.91 – 3.84 (1 H, m, (C-1)OC*H*H), 3.83 (3 H, s, NCH_3), 3.77 (1 H, dd, *J* 12.5, 2.3, H-6a), 3.75 – 3.65 (2 H, m, H-6b, (C-1)OCH*H*), 2.12 – 1.98 (2 H, m, $OCH_2CH_2CH_2N$); **^{13}C NMR** δ_C (101 MHz, Acetonitrile-d_3) 166.42, 166.32, 166.07 (3 C=O), 137.28 (NCHN), 134.65, 134.53, 130.39, 130.17, 130.15, 130.11, 129.97, 129.68, 129.61, 129.53 (C_{arom}), 124.56 (N*C*HCHN), 123.39 (NCH*C*HN), 101.21 (C-1), 75.37 (C-5), 74.57 (C-3), 73.10 (C-2), 70.26 (C-4), 66.56 ((C-1)O*C*H_2), 61.47 (C-6), 47.58 (NCH_2), 36.79 (NCH_3), 30.50 ($OCH_2CH_2CH_2N$); **^{19}F NMR** δ_F (377 MHz, Acetonitrile-d_3) -79.30 ($^-$OTf); **m/z** (TLC-MS+ (ESI)) $C_{34}H_{35}N_2O_9^+$ ([M - OTf]$^+$) calculated 615.2; found 615.0; (TLC-MS- (ESI)) $CF_3O_3S^-$ ([OTf]$^-$) calculated 148.9; found 149.1.

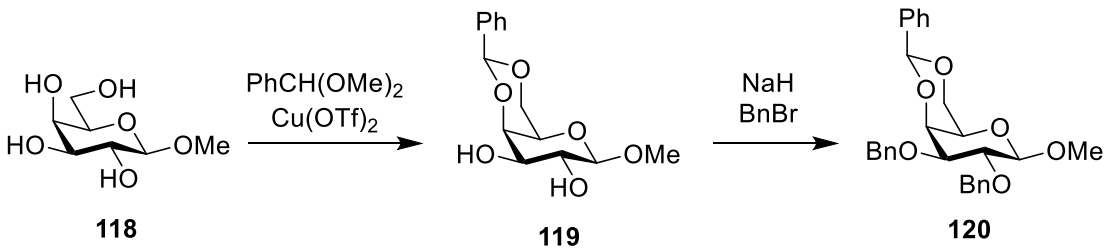
4-(1-Methyl-3-methyleneimidazolium)benzyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside trifluoromethanesulfonate 102



Following the general procedure for I-Tag glycosylations in flow using glycosyl acceptor 1-(4-(hydroxymethyl)benzyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate **157** (0.1762 g, 0.500 mmol, 1 eq) and glycosyl donor 2,3,4-tri-*O*-benzoyl-6-*O*-chloroacetyl-α-D-glucopyranosyl trichloroacetimidate **167** (0.7133 g, 1.000 mmol, 2 eq). 2.50 mL of anhydrous MeCN was added to the donor/acceptor vial,

resulting in a solution of volume 3.20 mL and therefore approximately 0.156 M in acceptor and 0.312 M in donor. To the other vial, 4.00 mL of anhydrous acetonitrile was added, followed by trimethylsilyl trifluoromethanesulfonate (43.4 μL, 0.240 mmol) to make a 0.06 M solution. The flow reaction was performed at 50 °C. Reaction solution was collected from the flow reactor directly into a solution of HCl, 1.25 M in MeOH (8.00 mL, 8.000 mmol), for a total of 47 min. The resulting acidic solution was stirred at RT for 16 h in air. After this time TLC-MS showed complete conversion to the desired product. The solution was diluted with DCM (15 mL) and water (15 mL). Product was extracted into the DCM phase, then the aqueous phase was washed with further DCM portions (2 x 15 mL) and all DCM portions were combined, dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. The crude residue was washed with Et_2O:DCM 9:1 (6 x 5 mL) and Et_2O:DCM 86:14 (10 mL) then dried under reduced pressure to yield the title compound **102** as an off-white solid (0.3674 g, 92 %) with spectroscopic details in accordance with the literature;[144] **^{1}H NMR** δ_H (400 MHz, Chloroform-*d*) 9.10 (1 H, s, NCHN), 7.97 – 7.88 (4 H, m, H_{arom}), 7.85 – 7.78 (2 H, m, H_{arom}), 7.56 – 7.48 (2 H, m, H_{arom}), 7.47 – 7.32 (5 H, m, H_{arom}) 7.30 – 7.18 (8 H, m, H_{arom}, NC*H*CHN, NCHC*H*N), 5.91 (1 H, t, *J* 9.7, H-3), 5.55 – 5.46 (2 H, m, H-2, H-4), 5.27 (2 H, s, NCH_2), 4.93 – 4.87 (2 H, m, H-1, (C-1)OC*H*H), 4.70 (1 H, d, *J* 12.5, (C-1)OCH*H*), 3.90 – 3.79 (5 H, m, H-5, H-6a, NCH_3), 3.71 (1 H, dd, *J* 12.3, 4.2, H-6b); **^{13}C NMR** δ_C (101 MHz, Chloroform-*d*) 166.18, 165.91, 165.40 (3 C=O), 138.68 (4° $C_{arom}CH_2O$(C-1)), 136.94 (NCHN), 133.84, 133.64, 133.43 (C_{arom}), 132.44 (4° $C_{arom}CH_2N$), 130.04, 129.87, 129.81, 129.23, 129.09, 128.95, 128.80, 128.67, 128.65, 128.63, 128.46 (C_{arom}), 123.75 (N*C*HCHN), 122.15 (NCH*C*HN), 100.49 (C-1), 74.83 (C-5), 72.93 (C-3), 72.10 (C-2), 70.83 ((C-1)O*C*H_2), 69.60 (C-4), 61.23 (C-6), 53.17 (NCH_2), 36.52 (NCH_3); **m/z** (TLC-MS+ (ESI)) $C_{39}H_{37}N_2O_9^+$ ([M - OTf]$^+$) calculated 677.2; found 677.1; (TLC-MS- (ESI)) $CF_3O_3S^-$ ([OTf]$^-$) calculated 149; found 149.

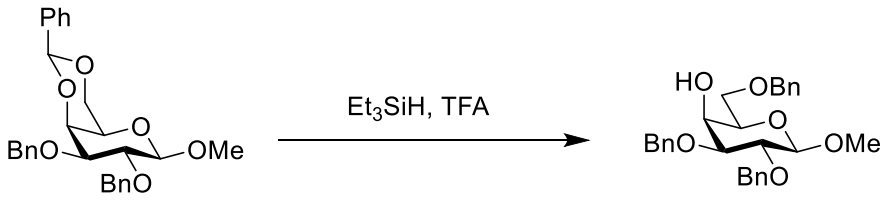
Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside 120



118 **119** **120**

Methyl-β-D-galactopyranoside **118** (4.85 g, 24.98 mmol) was dried under vacuum for 1 h in a flame-dried flask before being dissolved in anhydrous acetonitrile (100 mL) under a nitrogen atmosphere. Copper^{II} triflate (0.54 g, 1.49 mmol) and benzaldehyde dimethyl acetal (4.5 mL, 29.86 mmol) were added and the solution was sonicated under a nitrogen atmosphere for 70 min, after which time the reaction was quenched by addition of triethylamine (5 mL) and the solvent was removed under reduced pressure. Following purification by column chromatography (DCM:MeOH 19:1) methyl-4,6-*O*-benzylidene-β-D-galactopyranoside **119** was obtained as a white solid. The methyl-4,6-*O*-benzylidene-β-D-galactopyranoside **119** was dried under vacuum for 1 h in a flame-dried flask before being dissolved in anhydrous DMF (150 mL) under a nitrogen atmosphere. The solution was cooled to 0 ℃ and sodium hydride (60 % in mineral oil, 3.10 g, 77.50 mmol) was added. The reaction mixture was stirred for 15 min at 0 ℃ then 30 min at RT. The solution was cooled to 0 ℃ again and benzyl bromide (9.2 mL, 77.50 mmol) was added dropwise. After being left to stir under nitrogen for 18 h at RT, methanol (10 mL) was added to the reaction mixture, then the solvent was removed under reduced pressure. The residue was dissolved in DCM (250 mL), washed with water (2 x 120 mL) and brine (120 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure to give a crude product. Following purification by column chromatography (Hexane:EtOAc 9:1 → 7:3 → 1:1) the title compound **120** was obtained as a white solid (9.00 g, 78 %) with spectroscopic details in accordance with the literature;[108] **^{1}H NMR** δ_H (400 MHz, Chloroform-*d*) 7.61 – 7.52 (2 H, m, H_{arom}), 7.44 – 7.27 (13 H, m, H_{arom}), 5.50 (1 H, s, PhC(*H*)OO), 4.91 (1 H, d, *J* 10.9, PhCH_2), 4.82 – 4.72 (3 H, m, PhCH_2), 4.36 – 4.29 (2 H, m, H-6a, H-1), 4.12 (1 H, dd, *J* 3.7, 1.1, H-4), 4.03 (1 H, dd, *J* 12.3, 1.8, H-6b), 3.85 (1 H, dd, *J* 9.7, 7.6, H-2), 3.59 (3 H, s, OCH_3), 3.60 – 3.53 (1 H, m, H-3), 3.33 (1 H, q, *J* 1.5, H-5); **^{13}C NMR** δ_C (101 MHz, Chloroform-*d*) 139.04, 138.55, 137.96 (3 4° C_{arom}), 129.05, 128.46, 128.39, 128.24, 128.15, 127.88, 127.79, 127.64, 126.66 (15 C_{arom}), 104.85 (C-1), 101.49 (Ph*C*(H)OO), 79.30 (C-3), 78.58 (C-2), 75.36 (Ph*C*H_2), 74.08 (C-4), 72.13 (Ph*C*H_2), 69.36 (C-6), 66.53 (C-5), 57.19 (OCH_3); **m/z** (ESI-MS+) $C_{28}H_{30}O_6Na^+$ ([M + Na]$^+$) calculated 485.19; found 485.19.

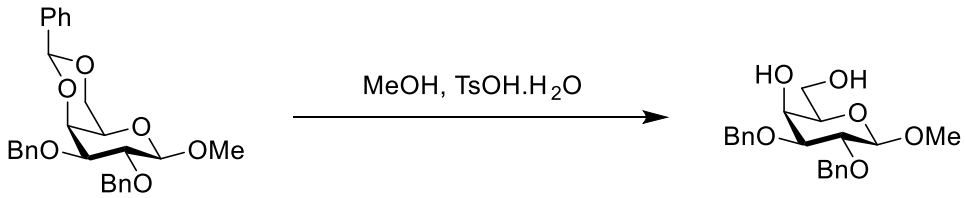
Methyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside 121



120 **121**

Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside **120** (10.68 g, 23.09 mmol) was dried under vacuum for 1 h in a flame-dried flask before being dissolved in anhydrous DCM (200 mL) under a nitrogen atmosphere. The solution was cooled to 0 ℃ and triethylsilane (18.5 mL, 115.45 mmol) was added. Trifluoroacetic acid (8.9mL, 115.45 mmol) was then added dropwise and the reaction mixture was left to stir at 0 ℃ for 30 min. When the reaction was judged to be complete by ^{1}H NMR spectroscopy, the reaction mixture was quenched by addition of triethylamine (37 mL) and methanol (31 mL). The organic phase was washed with water (130 mL) and brine (130 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure to give a crude product. Following purification by column chromatography (Hexane:EtOAc 9:1 → 7:3) the title compound **121** was obtained as a colourless oil (8.88 g, 83 %) with spectroscopic details in accordance with the literature;[108] **^{1}H NMR** δ_H (400 MHz, Chloroform-*d*) 7.43 – 7.24 (15 H, m, H_{arom}), 4.91 (1 H, d, *J* 11.1, PhC*H*H), 4.74 (1 H, d, *J* 11.3, PhCH*H*), 4.73 (2 H, s, PhCH_2), 4.61 (2 H, s, PhCH_2), 4.29 (1 H, d, *J* 7.7, H-1), 4.04 (1 H, d, *J* 3.3, H-4), 3.83 (1 H, dd, *J* 9.9, 6.0, H-6a), 3.76 (1 H, dd, *J* 9.9, 5.9, H-6b), 3.65 (1 H, dd, *J* 9.4, 7.7, H-2), 3.60 – 3.56 (1 H, m, H-5), 3.58 (3 H, s, OCH_3), 3.51 (1 H, dd, *J* 9.4, 3.4, H-3), 2.53 (1 H, s, OH); **^{13}C NMR** δ_C (101 MHz, Chloroform-*d*) 138.78, 138.12, 138.00 (3 4˚ C_{arom}), 128.56, 128.55, 128.41, 128.16, 127.99, 127.94, 127.89, 127.87, 127.70 (15 C_{arom}), 104.83 (C-1), 80.68 (C-3), 79.12 (C-2), 75.24 (Ph*C*H_2), 73.84 (Ph*C*H_2), 73.26 (C-5), 72.50 (Ph*C*H_2), 69.33 (C-6), 66.96 (C-4), 57.05 (OCH_3); **m/z** (ESI-MS+) $C_{28}H_{32}O_6Na^+$ ([M + Na]$^+$) calculated 487.2; found 487.2.

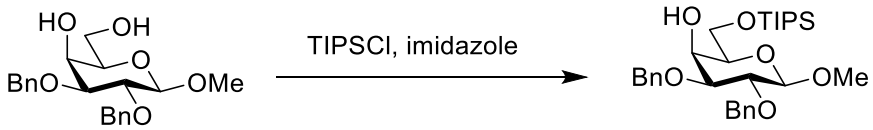
Methyl 2,3-di-*O*-benzyl-β-D-galactopyranoside 122



120 **122**

Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside **120** (9.00 g, 19.46 mmol), methanol (100 mL) and *p*-toluenesulfonic acid monohydrate (1.85 g, 9.73 mmol) were added to a flask and sonicated for 90 min, after which time triethylamine (8 mL) was added and the solvent was removed under reduced pressure. Following purification by column chromatography (Hexane:EtOAc 3:1 → 1:1 → EtOAc) the title compound **122** was obtained as an oil (6.20 g, 85 %, β:α ≈ 12:1), with spectroscopic details in accordance with the literature;[169] **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 7.40 – 7.27 (10 H, m, H_{arom}), 4.90 (1 H, d, *J* 11.0, PhC*H*H), 4.75 – 4.71 (3 H, m, PhCH_2), 4.30 (1 H, d, *J* 7.7, H-1), 4.00 (1 H, dd, *J* 3.4, 1.2, H-4), 3.98 (1 H, dd, *J* 11.7, 6.5, H-6a), 3.86 – 3.81 (1 H, m, H-6b), 3.64 (1 H, dd, *J* 9.4, 7.7, H-2), 3.58 (3 H, s, OCH_3), 3.51 (1 H, dd, *J* 9.3, 3.5, H-3), 3.47 (1 H, ddd, *J* 6.3, 4.7, 1.2, H-5), 2.66 (1 H, bs, C-4(OH)), 2.24 (1 H, bs, C-6(OH)); **^{13}C NMR** δ_C (126 MHz, Chloroform-d) 138.73, 137.88 (2 4° C_{arom}), 128.64, 128.45, 128.18, 128.11, 127.98, 127.76 (10 C_{arom}), 104.97 (C-1), 80.49 (C-3), 79.05 (C-2), 75.27 (PhCH_2), 74.02 (C-5), 72.71 (PhCH_2), 67.62 (C-4), 62.73 (C-6), 57.22 (OCH_3); **m/z** (ESI-MS+) $C_{21}H_{26}O_6Na^+$ ([M + Na]$^+$) calculated 397.16; found 397.16.

Methyl 2,3-di-*O*-benzyl-6-*O*-triisopropylsilyl-β-D-galactopyranoside 123

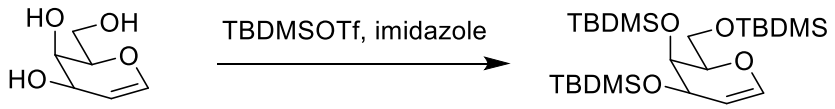


122 **123**

Methyl 2,3-di-*O*-benzyl-β-D-galactopyranoside **122** (6.20 g, 16.56 mmol) was dried under vacuum for 1 h in a flame-dried flask before being dissolved in anhydrous DMF (100 mL) under a nitrogen atmosphere. Imidazole (2.25 g, 33.12 mmol) was added and the resulting solution was cooled to 0 °C. To this solution TIPSCl (5.3 mL, 24.84 mmol) was added dropwise

and the resulting mixture was stirred at RT for 18 h under nitrogen, after which time the solvent was removed under reduced pressure. The residue was dissolved in DCM (150 mL) and washed with water (75 mL) and brine (75 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. Following purification by column chromatography (Hexane:EtOAc 99:1 → 9:1) the title compound **123** was obtained as a white solid (8.36 g, 95 %); **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 7.40 – 7.27 (10 H, m, H_{arom}), 4.90 (1 H, d, *J* 11.1, PhC*H*H), 4.76 – 4.72 (3 H, m, PhCH_2), 4.27 (1 H, d, *J* 7.8, H-1), 4.07 – 4.05 (1 H, m, H-4), 4.01 (1 H, dd, *J* 10.0, 6.5, H-6a), 3.91 (1 H, dd, *J* 10.0, 5.3, H-6b), 3.66 (1 H, dd, *J* 9.4, 7.7, H-2), 3.55 (3 H, s, OCH_3), 3.49 (1 H, dd, *J* 9.4, 3.3, H-3), 3.40 (1 H, t, *J* 6.0, H-5), 2.59 (1 H, d, *J* 1.9, OH), 1.14 – 1.00 (21 H, m, Si(^{i}Pr)$_3$); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) 138.94, 138.23 (2 4° C_{arom}), 128.58, 128.42, 128.16, 127.96, 127.94, 127.67 (10 C_{arom}), 104.93 (C-1), 80.90 (C-3), 79.32 (C-2), 75.25 (Ph*C*H_2), 74.56 (C-5), 72.52 (Ph*C*H_2), 66.70 (C-4), 62.67 (C-6), 56.96 (OCH_3), 18.09, 18.06, 12.04 (9 C(^{i}Pr)); **m/z** (ESI-HRMS) $C_{30}H_{46}O_6SiNa^+$ ([M + Na]$^+$) calculated 553.2956; found 553.2934; $C_{29}H_{43}O_5Si^+$ ([M – OMe]$^+$) calculated 499.2874; found 499.2851; **IR** ν_{max}/cm^{-1} 3490br, 2941, 2865, 1454, 1383, 1202, 1100, 1074, 882, 806, 735, 670; $[\alpha]_D^{22}$ + 50 [*c* 0.94, $CHCl_3$].

Tri-*O*-(*tert*-butyldimethylsilyl)-D-galactal 125

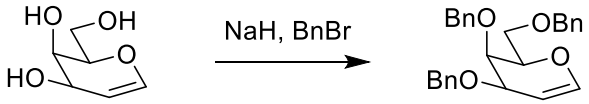


124 **125**

D-Galactal **124** (0.50 g, 3.42 mmol) was dried under vacuum for 1 h in a flame-dried flask before being dissolved in anhydrous DMF (5 mL) under a nitrogen atmosphere. Imidazole (2.10 g, 30.85 mmol) was added, followed by *tert*-butyldimethylsilyl trifluoromethanesulfonate (4.7 mL, 20.52 mmol) dropwise and the reaction was left to stir under a nitrogen atmosphere for 25 h at 50 °C. The reaction mixture was then poured over hexane (20 mL) and crushed ice. The aqueous phase was washed with hexane (2 x 10 mL) and hexane fractions were combined. The hexane phase was washed with water (2 x 10 mL) and brine (2 x 10 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. Following purification by column chromatography (Hexane:DCM 9:1 +

1 % NEt_3), the title compound **125** was obtained as a colourless syrup (1.45 g, 87 %) with spectroscopic details in accordance with the literature;[108] **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 6.21 (1 H, dd, *J* 6.2, 0.8, H-1), 4.65 (1 H, app s, H-2), 4.20 – 3.97 (4 H, m, H-3, H-4, H-5, H-6a), 3.86 (1 H, d, *J* 8.3, H-6b), 0.91 (9 H, s, $C(CH_3)_3$), 0.90 (9 H, s, $C(CH_3)_3$), 0.90 (9 H, s, $C(CH_3)_3$), 0.10 (3 H, s, $SiCH_3$), 0.10 (3 H, s, $SiCH_3$), 0.07 (3 H, s, $SiCH_3$), 0.07 (3 H, s, $SiCH_3$), 0.05 (6 H, s, $SiCH_3$); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) 142.82 (C-1), 102.80 (C-2), 79.73, 69.33, 65.28 (C-3, C-4, C-5), 61.07 (C-6), 26.19, 26.13, 26.05 (9 $C(CH_3)_3$), 18.61, 18.42, 18.35 (3 $C(CH_3)_3$), -4.05, -4.23, -4.60, -4.77, -4.98, -5.09 (6 $SiCH_3$); **m/z** (ESI-MS+) $C_{24}H_{52}O_4Si_3Na^+$ ($[M + Na]^+$) calculated 511.31; found 511.31; $C_{18}H_{37}O_3Si_2^+$ ($[M - OTBDMS]^+$) calculated 357.23; found 357.23.

Tri-*O*-benzyl-D-galactal 126

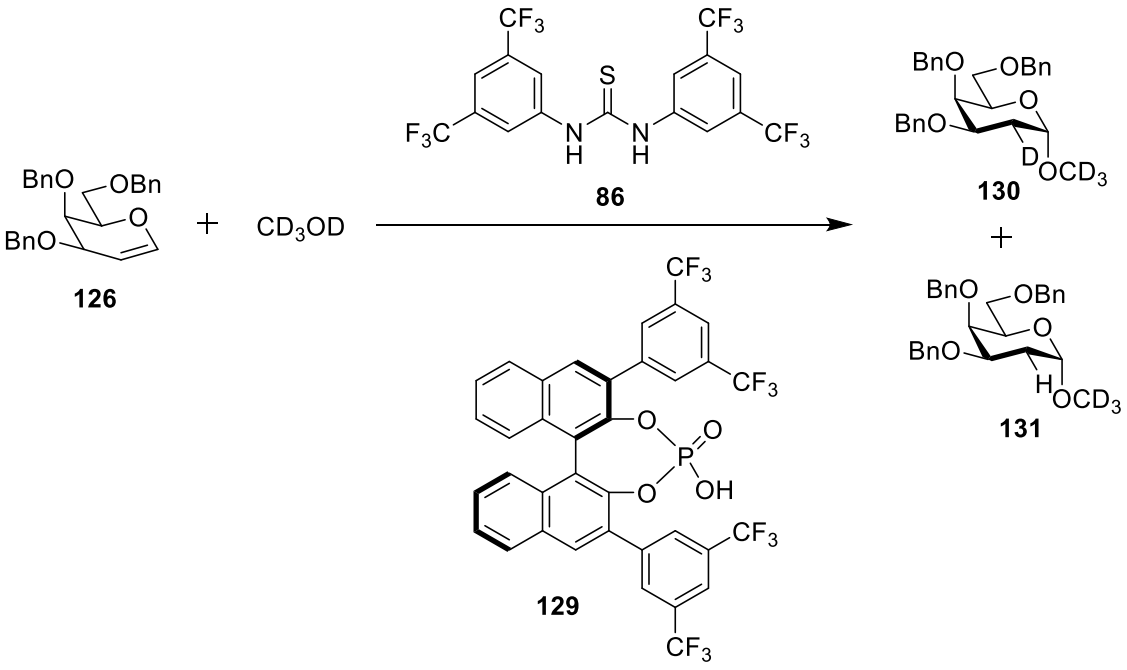


124 **126**

D-Galactal **124** (4.00 g, 27.37 mmol) was dried under vacuum for 30 min in a flame-dried flask before being dissolved in anhydrous DMF (200 mL) and cooled to 0 °C under a nitrogen atmosphere. Sodium hydride (60 % in mineral oil, 6.57 g, 164.23 mmol) was added and the mixture stirred for 30 min at RT, after which time the mixture was again cooled to 0 °C. Benzyl bromide (19.5 mL, 164.23 mmol) was added dropwise and the reaction mixture was stirred for 16.5 h at RT, after which time methanol (20 mL) was added. Solvent was removed under reduced pressure and the residue was dissolved in DCM (100 mL). The resulting solution was washed with water (100 mL), then the aqueous phase was washed with DCM (2 x 50 mL) and the organic washings were combined. The combined DCM phase was washed with brine (100 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. The crude residue was purified by column chromatography (Hexane:EtOAc 20:1 → 15:1 → 10:1). To remove amine impurity **127** that co-eluted with the title compound during column chromatography, the residue was dissolved in hexane (100 mL) and washed with 1 M $HCl_{(aq.)}$ (2 x 100 mL), $NaHCO_{3\ (sat.\ aq.)}$ (100 mL) and water (100 mL). The hexane phase was dried using magnesium sulfate, filtered and the solvent was removed

under reduced pressure to yield the title compound **126** as a colourless syrup (7.63 g, 67 %) with spectroscopic details in accordance with the literature;[170] **^{1}H NMR** δ_H (400 MHz, Chloroform-*d*) 7.38 – 7.27 (15 H, m, H_{arom}), 6.38 (1 H, dd, *J* 6.2, 1.5, H-1), 4.92 – 4.84 (2 H, m, H-2, PhC*H*H), 4.70 – 4.59 (3 H, m, PhCH_2), 4.52 (1 H, d, *J* 11.9, PhC*H*H), 4.44 (1 H, d, *J* 11.9, PhCH*H*), 4.23 – 4.16 (2 H, m, H-3, H-5), 3.99 – 3.93 (1 H, m, H-4), 3.79 (1 H, dd, *J* 10.2, 7.2, H-6a), 3.66 (1 H, dd, *J* 10.1, 5.1, H-6b); **^{13}C NMR** δ_C (101 MHz, Chloroform-*d*) 144.33 (C-1), 138.65, 138.51, 138.14 (3 4° C_{arom}), 128.52, 128.46, 128.28, 128.03, 127.83, 127.69, 127.58 (C_{arom}), 100.10 (C-2), 75.83 (C-5), 73.55 (Ph*C*H_2), 73.45(Ph*C*H_2), 71.42 (C-4), 71.02 (Ph*C*H_2), 70.88 (C-3), 68.57 (C-6); **m/z** (ESI-MS+) $C_{27}H_{28}O_4Na^+$ ([M + Na]$^+$) calculated 439.2; found 439.2.

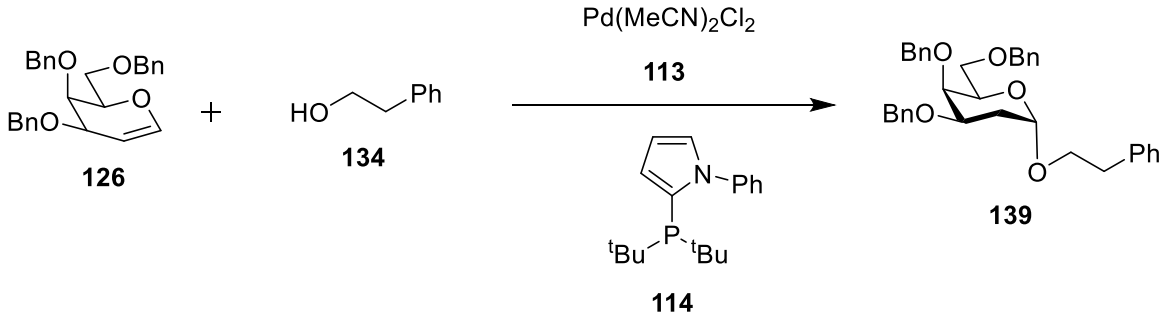
d_3-Methyl 2-deoxy-3,4,6-tri-*O*-benzyl-2-d-α-D-lyxo-hexapyranoside 130 and d_3-methyl 2-deoxy-3,4,6-tri-*O*-benzyl-α-D-lyxo-hexapyranoside 131



In a flame-dried microwave vial donor tri-*O*-benzyl-D-galactal **126** (0.1000 g, 0.240 mmol) was dried under vacuum for 16 h. In a separate flame-dried microwave vial, N,N'−bis(3,5−bis(trifluoromethyl)phenyl)-thiourea **86** (0.0120 g, 0.0240 mmol) and (R)-3,3'-bis(3,5-bis(trifluoromethyl)phenyl)-1,1'-binaphthyl-2,2'-diyl hydrogenphosphate **129** (0.0185 g, 0.024 mmol) were also dried under vacuum for 16 h. Anhydrous DCM (2 mL) was added to the thiourea-acid vial and the resulting solution was stirred at RT under a nitrogen

atmosphere for 30 min. After this time, methanol-d_4 (8.1 µL, 0.199 mmol) which had been dried using activated 3Å molecular sieves, was added to the thiourea-acid solution. The resulting solution was then added to the vial containing the glycosyl donor. The reaction was stirred at RT under a nitrogen atmosphere for 3 h 15 min, after which time TLC (Hexane:EtOAc 7:3) showed the reaction to be complete. Solvent was then removed under reduced pressure. Following column chromatography (Hexane:EtOAc 10:1) the title compounds **130** and **131** were obtained as an inseparable isotopic mixture (0.0712 g, 79 % combined yield) in a ratio of **130**:**131** = 54:46 as determined by ^{1}H NMR spectroscopy by comparison of the H-2 peaks. Spectroscopic details were in accordance with the literature for previously reported compound **130** and the isotopologue of **131**, methyl 2-deoxy-3,4,6-tri-*O*-benzyl-α-D-lyxo-hexapyranoside;[150] **^{1}H NMR** δ_H (400 MHz, Chloroform-*d*) 7.42 – 7.24 (15 H, m, H_{arom}), 4.96 (1 H, d, *J* 11.6, PhC*H*H), 4.90 (1 H, app d, *J* 3.8, H-1), 4.66 (1 H, d, *J* 11.6, PhCH*H*), 4.62 (2 H, s, PhCH_2), 4.55 (1 H, d, *J* 11.8, PhC*H*H), 4.46 (1 H, d, *J* 11.7, PhCH*H*), 4.00 – 3.87 (3 H, m, H-3, H-4, H-5), 3.65 – 3.61 (2 H, m, H-6a, H-6b), 2.32 – 2.18 (1 H, m, H-2ax **130** and **131**), 2.03 (1 H, ddt, *J* 12.7, 4.5, 1.3, H-2eq **131**); **^{13}C NMR** δ_C (101 MHz, Chloroform-*d*) 138.92, 138.65, 138.18 (3 4° C_{arom}), 128.51, 128.50, 128.37, 128.33, 127.90, 127.80, 127.65, 127.40 (C_{arom}), 98.98 (C-1), 74.77 (app d, C-3), 74.43 (Ph*C*H_2), 73.60 (Ph*C*H_2), 73.17 (app d, C-4), 70.54 (Ph*C*H_2), 69.89 (C-5), 69.76 (C-6), 31.26 (C-2 **131**), 30.81 (app d, C-2 **130**); **m/z** (ESI-HRMS) **130** $C_{28}H_{29}D_4O_5^+$ ([M + H]$^+$) calculated: 453.2574; found 453.2577; $C_{28}H_{28}D_4O_5Na^+$ ([M + Na]$^+$) calculated: 475.2393; found 475.2386; **131** $C_{28}H_{30}D_3O_5^+$ ([M + H]$^+$) calculated: 452.2511; found 452.2521; $C_{28}H_{29}D_3O_5Na^+$ ([M + Na]$^+$) calculated: 474.2330; found 474.2334.

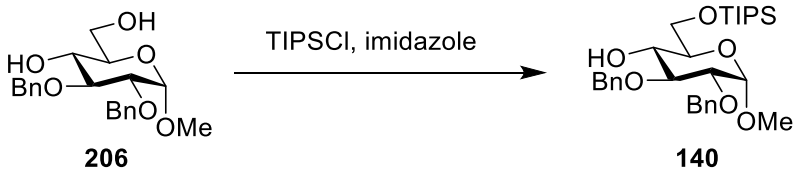
Phenethyl 3,4,6-tri-*O*-benzyl-α-D-lyxo-hexapyranoside 139



Following the general procedure for palladium catalysed glycosylation reactions, glycosyl donor tri-*O*-benzyl-D-galactal **126** (0.100 g, 0.240 mmol), glycosyl acceptor phenethyl

alcohol **134** (0.022 g, 0.180 mmol), metal catalyst bis(acetonitrile)dichloropalladium (II) **113** (0.019 g, 0.072 mmol) and ligand 2-(di-*tert*-butylphosphanyl)-1-phenyl-1H-pyrrole **114** (0.021 g, 0.072 mmol) were dissolved in 2 mL of anhydrous DCM. The reaction mixture was stirred for 18 h before being quenched. Following purification by column chromatography (Hexane:EtOAc 6:1), in which α and β anomers were separated, the title compound **139** was obtained as a pale yellow oil (0.067 g, 69 %); **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 7.40 – 7.14 (20 H, m, H_{arom}), 4.98 (1 H, app d, *J* 3.5, H-1), 4.92 (1 H, d, *J* 11.6, PhC*H*HO), 4.61 (1 H, d, *J* 11.2, PhCH*H*O), 4.60 (2 H, s, PhCH_2O), 4.47 (1 H, d, *J* 11.8, PhC*H*HO), 4.39 (1 H, d, *J* 11.8, PhCH*H*O), 3.89 (1 H, ddd, *J* 12.0, 4.6, 2.5, H-3), 3.86 (1 H, app s, H-4), 3.85 – 3.79 (1 H, m, PhCH_2C*H*HO), 3.70 (1 H, t, *J* 6.5, H-5), 3.61 (1 H, dt, *J* 9.8, 6.7, PhCH_2CH*H*O), 3.56 (1 H, dd, *J* 9.4, 6.7, H-6a), 3.51 (1 H, dd, *J* 9.3cc, 6.1, H-6b), 2.86 (2 H, t, *J* 7.0, PhCH_2CH_2O), 2.21 (1 H, td, *J* 12.2, 3.7, H-2ax), 1.98 (1 H, app dd, *J* 12.5, 4.4, H-2eq); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) 139.21 (4˚ C_{arom}CH_2CH_2O), 139.07, 138.71, 138.31 (3 4˚ C_{arom}CH_2O), 129.08, 128.54, 128.48, 128.40, 128.33, 128.32, 127.85, 127.74, 127.66, 127.59, 127.49, 126.31 (16 C_{arom}), 97.80 (C-1), 74.78 (C-3), 74.38 (Ph*C*H_2O), 73.51 (Ph*C*H_2O), 73.17 (C-4), 70.49 (Ph*C*H_2O), 69.95 (C-5), 69.71 (C-6), 68.12 (PhCH_2*C*H_2O), 36.33 (Ph*C*H_2CH_2O), 31.33 (C-2); **m/z** (ESI-HRMS) $C_{35}H_{38}O_5Na^+$ ([M + Na]$^+$) calculated 561.2611; found 561.2596; **IR** ν_{max}/cm^{-1} 3063, 3029, 2919, 2866, 1497, 1454, 1359, 1207, 1095, 1062, 1028, 959, 735, 697; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{23}}$ + 63 [*c* 0.65, DCM].

Methyl 2,3-di-*O*-benzyl-6-*O*-triisopropylsilyl-α-D-glucopyranoside 140

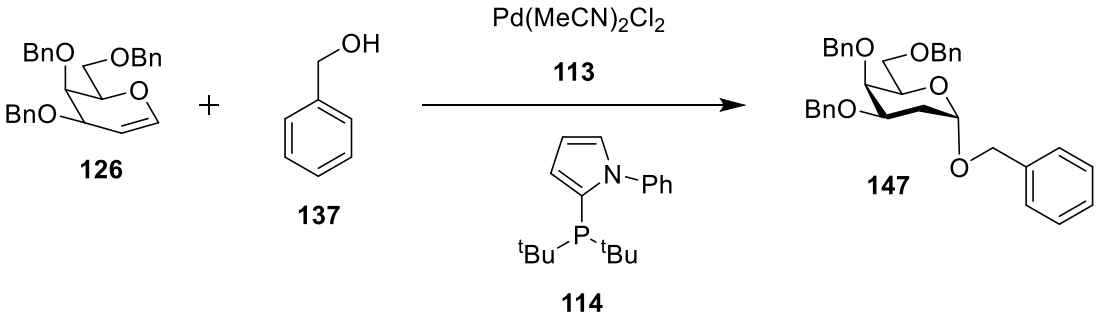


206 **140**

Methyl 2,3-di-*O*-benzyl-α-D-glucopyranoside **206** (0.80 g, 2.14 mmol) was dried under vacuum for 1 h in a flame-dried flask before being dissolved in anhydrous DMF (12 mL) under a nitrogen atmosphere. Imidazole (0.29 g, 4.28 mmol) and TIPSCl (0.67 mL, 3.21 mmol) were added and the resulting mixture stirred at RT for 20 h under nitrogen, after which time the solvent was removed under reduced pressure. The residue was dissolved in DCM (9 mL) and washed with water (5 mL) and brine (5 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. Following purification by column

chromatography (Petroleum ether (40-60):EtOAc 9:1) and drying under vacuum for 3 h the title compound **140** was obtained as a colourless oil (0.58 g, 51 %) with spectroscopic details in accordance with the literature;[108] **^{1}H NMR** δ_H (301 MHz, Chloroform-*d*) 7.43 – 7.27 (10 H, m, H_{arom}), 4.98 (1 H, d, *J* 11.3, PhC*H*H), 4.81 (1 H, d, *J* 11.4, PhCH*H*), 4.78 (1 H, d, *J* 12.0, PhC*H*H), 4.66 (1 H, d, *J* 12.1, PhCH*H*), 4.61 (1 H, d, *J* 3.5, H-1), 3.88 (2 H, d, *J* 4.8, H-6a, H-6b), 3.83 (1 H, dd, *J* 9.6, 8.4, H-3), 3.69 – 3.52 (2 H, m, H-4, H-5), 3.49 (1 H, dd, *J* 9.4, 3.7, H-2), 3.39 (3 H, s, OCH_3), 2.78 (1 H, s, OH), 1.18 – 1.00 (21 H, m, TIPS); **^{13}C NMR** δ_C (76 MHz, Chloroform-*d*) 139.08, 138.33 (2 4° C_{arom}), 128.63, 128.57, 128.20, 128.14, 128.00, 127.84 (10 C_{arom}), 98.14 (C-1), 81.65 (C-3), 79.65 (C-2), 75.64 (Ph*C*H_2), 73.32 (Ph*C*H_2), 72.72 (C-4), 70.78 (C-5), 64.84 (C-6), 55.18 (OCH_3), 18.06 (SiCH(*C*H_3)$_2$), 11.99 (Si*C*H(CH_3)$_2$); **m/z** (ESI-MS+) $C_{30}H_{46}O_6SiNa^+$ ([M + Na]$^+$) calculated 553.30; found 553.29.

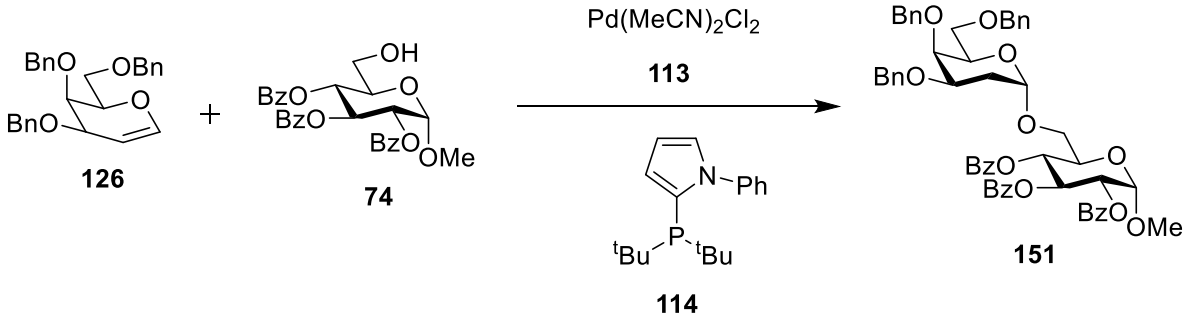
Benzyl 3,4,6-tri-*O*-benzyl-α-D-lyxo-hexapyranoside 147



Following the general procedure for palladium catalysed glycosylation reactions, glycosyl donor tri-*O*-benzyl-D-galactal **126** (0.100 g, 0.240 mmol), glycosyl acceptor benzyl alcohol **137** (0.020 g, 0.180 mmol), metal catalyst bis(acetonitrile)dichloropalladium (II) **113** (0.019 g, 0.072 mmol) and ligand 2-(di-*tert*-butylphosphanyl)-1-phenyl-1H-pyrrole **114** (0.021 g, 0.072 mmol) were dissolved in 2 mL of anhydrous DCM. The reaction mixture was stirred for 17 h before being quenched. Following purification by column chromatography (Hexane:EtOAc 9:1) the title compound **147** was obtained as a colourless oil (0.091 g, 96 %) with spectroscopic details in accordance with the literature;[171] **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 7.37 – 7.22 (20 H, m, H_{arom}), 5.08 (1 H, app d, *J* 3.5, H-1), 4.93 (1 H, d, *J* 11.6, PhC*H*H), 4.67 (1 H, d, *J* 11.9, PhC*H*H), 4.62 (1 H, d, *J* 11.7, PhCH*H*), 4.59 (2 H, d, *J* 1.3, Ph*C*H_2), 4.50 (1 H, d, *J* 11.8, PhC*H*H), 4.47 (2 H, d, *J* 11.9, PhCH*H*), 4.43 (1 H, d, *J* 11.8, PhCH*H*), 3.99 (1 H, ddd, *J* 12.0, 4.6, 2.5, H-3), 3.96 (1 H, t, *J* 6.7, H-5), 3.94 (1 H, bs, H-4), 3.61 (1 H, dd, *J* 9.4,

6.8, H-6a), 3.56 (1 H, dd, *J* 9.4, 6.0, H-6b), 2.25 (1 H, td, *J* 12.4, 3.8, H-2ax), 2.07 – 2.01 (1 H, m, H-2eq); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) 139.05, 138.68, 138.29, 138.02 (4 4° C_{arom}), 128.54, 128.52, 128.51, 128.37, 128.35, 128.09, 127.91, 127.81, 127.77, 127.65, 127.63, 127.48 (20 C_{arom}), 97.26 (H-1), 75.02 (C-3), 74.44 (Ph*C*H_2), 73.63 (Ph*C*H_2), 73.17 (C-4), 70.65 (Ph*C*H_2), 70.25 (C-5), 69.74 (C-6), 69.06 (Ph*C*H_2), 31.30 (C-2); **m/z** (ESI-MS+) $C_{34}H_{36}O_5Na^+$ ([M + Na]$^+$) calculated 547.2; found 547.2.

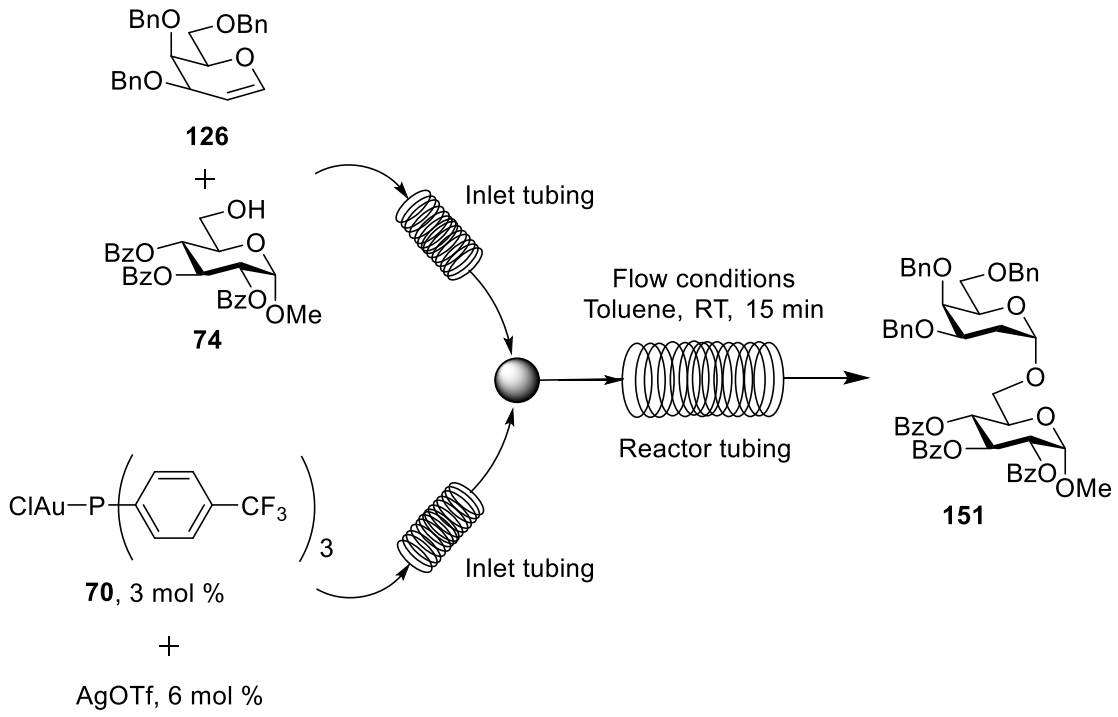
Methyl 2,3,4-tri-*O*-benzoyl-6-*O*-(3,4,6-tri-*O*-benzyl-α-D-lyxo-hexapyranosyl)-α-D-glucopyranoside 151



Following the general procedure for palladium catalysed glycosylation reactions, glycosyl donor tri-*O*-benzyl-D-galactal **126** (0.100 g, 0.240 mmol), glycosyl acceptor methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside **74** (0.091 g, 0.180 mmol), metal catalyst bis(acetonitrile)dichloropalladium (II) **113** (0.019 g, 0.072 mmol) and ligand 2-(di-*tert*-butylphosphanyl)-1-phenyl-1H-pyrrole **114** (0.021 g, 0.072 mmol) were dissolved in 2 mL of anhydrous DCM. The reaction mixture was stirred for 18 h before being quenched. Following purification by column chromatography (Hexane:EtOAc 6:1 → 4:1) the title compound **151** was obtained as a pale yellow oil (0.136 g, 82 %, α:β >30:1), with spectroscopic details in accordance with the literature;[108] **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 8.03 – 7.98 (2 H, m, H_{arom}), 7.97 – 7.92 (2 H, m, H_{arom}), 7.91 – 7.86 (2 H, m, H_{arom}), 7.56 – 7.48 (1 H, m, H_{arom}), 7.49 – 7.44 (1 H, m, H_{arom}), 7.44 – 7.34 (7 H, m, H_{arom}), 7.34 – 7.23 (13 H, m, H_{arom}), 7.23 – 7.18 (2 H, m, H_{arom}), 6.14 (1 H, t, *J* 9.9, H-3), 5.66 (1 H, t, *J* 9.9, H-4), 5.30 (1 H, dd, *J* 10.2, 3.7, H-2), 5.22 (1 H, d, *J* 3.6, H-1), 5.02 (1 H, app d, *J* 3.3, H-1'), 4.91 (1 H, d, *J* 11.6, PhC*H*H), 4.59 (1 H, d, *J* 11.6, PhCH*H*), 4.57 (2 H, s, PhCH_2), 4.34 (1 H, d, *J* 12.0, PhC*H*H), 4.26 (1 H, d, *J* 12.0, PhCH*H*), 4.21 (1 H, ddd, *J* 10.2, 4.4, 3.1, H-5), 3.97 (1 H, ddd, *J* 12.0, 4.6, 2.4, H-3'), 3.89 (1 H, s, H-4'), 3.88 (1 H, dd, *J* 11.3, 4.4, H-6a), 3.89 – 3.83 (1 H, m, H-5'), 3.60 (1 H, dd, *J* 11.1, 3.1, H-6b), 3.42

(2 H, t, *J* 6.2, H-6a', H-6b'), 3.41 (3 H, s, OCH_3), 2.20 (1 H, td, *J* 12.4, 3.7, H-2ax'), 2.00 (1 H, app dd, *J* 12.7, 4.7, H-2eq'); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) 165.97, 165.94, 165.39 (3 C=O), 139.03, 138.73, 138.34 (3 4° C_{arom}(Bn)), 133.46, 133.37, 133.15 (3 C_{arom} (Bz)), 130.06, 129.89, 129.78 (6 C_{arom} (Bz)), 129.44, 129.32, 129.23 (3 4° C_{arom}(Bz)), 128.52, 128.50, 128.47, 128.38, 128.30, 128.27, 127.63, 127.59, 127.58, 127.55, 127.54 (18 C_{arom}), 98.23 (C-1'), 97.11 (C-1), 74.57 (C-3'), 74.38 (Ph*C*H_2), 73.28 (Ph*C*H_2), 73.15 (C-4'), 72.19 (C-2), 70.85 (C-3), 70.53 (Ph*C*H_2), 70.01 (C-5'), 69.63 (C-4), 69.51 (C-6'), 68.30 (C-5), 65.87 (C-6), 55.59 (OCH_3), 31.04 (C-2'); **m/z** (ESI-MS+) $C_{55}H_{54}O_{13}Na^+$ ([M + Na]$^+$) calculated 945.3; found 945.3.

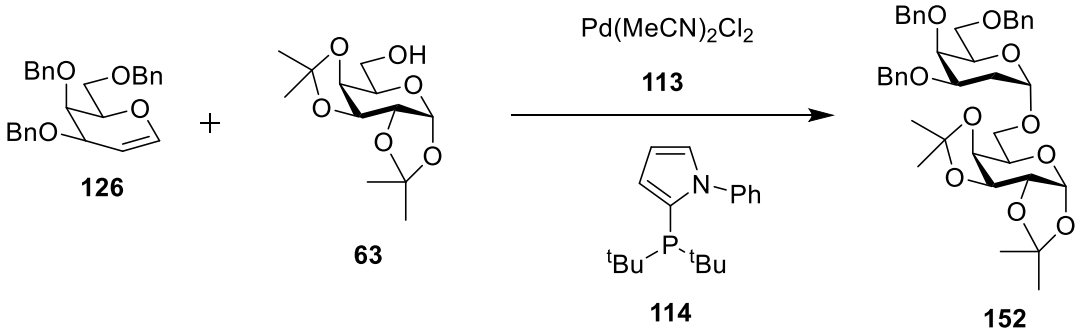
Methyl 2,3,4-tri-*O*-benzoyl-6-*O*-(3,4,6-tri-*O*-benzyl-α-D-lyxo-hexapyranosyl)-α-D-glucopyranoside 151



Glycosyl acceptor methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside **74** (0.0406 g, 0.0800 mmol, 1 eq) and glycosyl donor tri-*O*-benzyl-D-galactal **126** (0.0500 g, 0.1200 mmol, 1.5 eq) were placed in a dry vial and dried under vacuum for 30 min. Gold catalyst chloro[tris(*para*-trifluoromethylphenyl)phosphine]gold (I) **70** (0.0016 g, 0.0023 mmol, 0.03 eq) and silver triflate (0.0012 g, 0.0047 mmol, 0.06 eq) were placed in another dry vial and dried under vacuum for 30 min. After this drying period, 0.4 mL of anhydrous toluene was added to each vial, to make a donor/acceptor solution that is approximately 0.200 M in

acceptor **74** and 0.300 M in donor **126**, and a catalyst solution that is approximately 0.006 M in gold catalyst **70** and 0.012 M in silver triflate. The catalyst solution was sonicated for 1 minute under nitrogen to aid dissolution. The flow reactor, consisting of two inlet tubes (each with internal volume = 0.2027 mL), joining at a T-junction leading to a 5 m reactor tube (internal volume = 2.451 mL) was flushed with anhydrous toluene. Equal volumes (0.20 mL) of the two reactant solutions were taken and injected into the two inlet tubing pieces of the reactor via a double syringe pump. Anhydrous toluene syringes were then connected to the reactor and the solvent was used to push the reacting solutions through the reactor tubing, setting the desired flow rate corresponding to the residence time (15 minutes, 0.0817 mL/min in each syringe for a combined flow rate of 0.1634 mL/min in reactor tubing). The mixture that flowed from the microreactor was dropped in a flask containing triethylamine to quench the reaction. Solution from the flow reactor was collected for a total of 45 min to ensure that all product was collected. The reaction mixture solvent was removed under reduced pressure and the crude product was purified by column chromatography (Hexane:EtOAc 9:1 → 4:1). The title compound **151** was obtained as a pale-yellow oil (0.0328 g, 89 %), with spectroscopic details as described previously in this document.

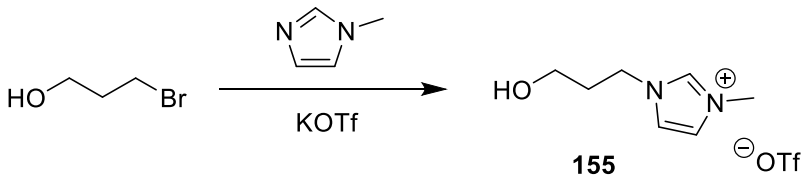
6-*O*-(3,4,6-Tri-*O*-benzyl-α-D-lyxo-hexapyranosyl)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranoside 152



Following the general procedure for palladium catalysed glycosylation reactions, glycosyl donor tri-*O*-benzyl-D-galactal **126** (0.100 g, 0.240 mmol), glycosyl acceptor 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose **63** (0.047 g, 0.180 mmol), metal catalyst bis(acetonitrile)dichloropalladium (II) **113** (0.019 g, 0.072 mmol) and ligand 2-(di-*tert*-butylphosphanyl)-1-phenyl-1H-pyrrole **114** (0.021 g, 0.072 mmol) were dissolved in 2 mL of anhydrous DCM. The reaction mixture was stirred for 42 h before being quenched. Following

purification by column chromatography (Hexane:EtOAc 8:1 → 5:1) during which α and β anomers were separated, the title compound **152** was obtained as a colourless oil (0.044 g, 36 %) with spectroscopic details in accordance with the literature;[108] **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 7.39 – 7.27 (15 H, m, H_{arom}), 5.54 (1 H, d, *J* 5.0, H-1), 5.06 (1 H, app d, *J* 3.0, H-1'), 4.94 (1 H, d, *J* 11.6, PhC*H*H), 4.64 (1 H, d, *J* 11.6, PhCH*H*), 4.64 – 4.59 (3 H, m, H-3, PhCH_2), 4.51 (1 H, d, *J* 11.8, PhC*H*H), 4.45 (1 H, d, *J* 11.8, PhCH*H*), 4.33 (1 H, dd, *J* 5.0, 2.4, H-2), 4.24 (1 H, dd, *J* 7.9, 1.9, H-4), 4.01 – 3.95 (4 H, m, H-3', H-4', H-5', H-5), 3.77 (1 H, dd, *J* 10.7, 6.8, H-6a'), 3.69 (1 H, dd, *J* 10.7, 6.4, H-6b'), 3.64 (1 H, dd, *J* 9.2, 7.4, H-6a), 3.57 (1 H, dd, *J* 9.2, 5.6, H-6b), 2.28 – 2.21 (1 H, m, H-2ax'), 2.08 – 2.02 (1 H, m, H-2eq'), 1.54 (3 H, s, C(CH_3)), 1.45 (3 H, s, (C(CH_3)), 1.35 (6 H, s C(CH_3)); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) 139.07, 138.74, 138.27 (3 4° C_{arom}), 128.50, 128.36, 128.31, 127.95, 127.76, 127.59, 127.44 (15 C_{arom}), 109.44, 108.66 (2 *C*$(CH_3)_2$), 97.66 (C-1'), 96.49 (C-1), 74.84 (CH), 74.45 (Ph*C*H_2), 73.52 (Ph*C*H_2), 73.02 (CH), 71.22 (C-4), 70.81, 70.76 (C-2, C-3), 70.55 (Ph*C*H_2), 69.96 (CH), 69.33 (C-6), 65.98 (CH), 65.67 (C-6'), 31.27 (C-2'), 26.27, 26.12, 25.09, 24.69 (4 C(*C*H_3)); **m/z** (ESI-MS+) $C_{39}H_{48}O_{10}Na^+$ ($[M + Na]^+$) calculated 699.3; found 699.3.

1-(3-Hydroxypropyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate 155

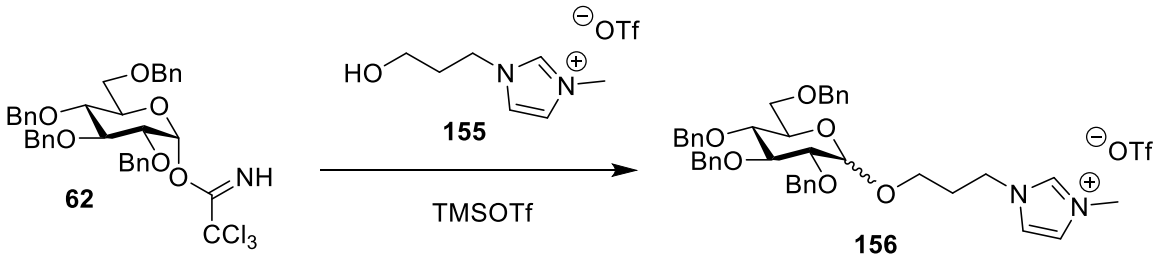


155

3-Bromopropanol (5.00 mL, 55.40 mmol), 1-methylimidazole (13.25 mL, 166.20 mmol), potassium trifluoromethanesulfonate (20.85 g, 110.80 mmol) and anhydrous acetonitrile (100 mL) were added to an oven-dried flask. The resulting mixture was heated under reflux at 80 °C under a N_2 atmosphere for 21 h. ^{1}H NMR analysis of the crude material revealed that the alcohol starting material had been converted to the product quantitatively. The reaction mixture was filtered and the solvent was removed under reduced pressure. The crude product was heated to 80 °C under vacuum (0.6 mbar) overnight and subsequently triturated five times (Et_2O:DCM 13:7). To remove final traces of 1-methylimidazole, a 0.50 g portion of the product was purified by reverse phase HPLC (Water:MeCN) and freeze-dried, to afford the title compound **155** (0.40 g, 80 %) as a colourless oil, with spectroscopic details

in accordance with the literature;[172] **^{1}H NMR** δ_H (400 MHz, Chloroform-*d*) 8.48 (1 H, s, NCHN), 7.41 (1 H, t, *J* 1.8, NC*H*CHN), 7.35 (1 H, t, *J* 1.8, NCHC*H*N), 4.26 (2 H, t, *J* 7.0, CH_2), 3.84 (3 H, s, CH_3), 3.54 (2 H, t, *J* 5.9, CH_2), 2.41 (1 H, bs, OH), 2.05 – 1.98 (2 H, m, CH_2); **^{13}C NMR** δ_C (101 MHz, Chloroform-*d*) 137.20 (NCHN), 124.59, 123.51 (2 NCHCHN), 58.48 (CH_2), 47.74 (CH_2), 36.85 (CH_3), 33.09 (CH_2); **^{19}F NMR** δ_F (377 MHz, Chloroform-*d*) -74.04 ($^-$OTf); **m/z** (ESI-MS+) $C_7H_{13}N_2O^+$ ([M – OTf]$^+$) calculated: 141.1; found 141.1.

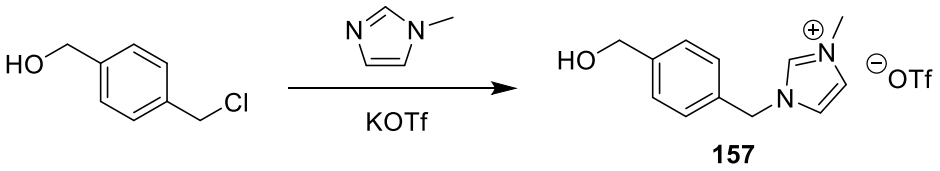
3-(3-Methylimidazolium)-1-propyl 2,3,4,6-tetra-*O*-benzyl-D-glucopyranoside trifluoromethanesulfonate 156



Following the general procedure for I-Tag glycosylations in flow using glycosyl acceptor 1-(3-hydroxypropyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate **155** (0.0581 g, 0.200 mmol, 1 eq) and glycosyl donor 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl trichloroacetimidate **62** (0.2740 g, 0.400 mmol, 2.0 eq). 1.0 mL of anhydrous acetonitrile was added to the donor/acceptor vial, resulting in a solution of volume 1.20 mL and therefore approximately 0.167 M in acceptor and 0.333 M in donor. To the other vial, 1.5 mL of anhydrous acetonitrile was added, followed by trimethylsilyl trifluoromethanesulfonate (16.3 μL, 0.090 mmol) to make a 0.06 M solution. Reaction solution was collected for a total of 12 min 45 sec into reagent grade DCM. The crude product was washed with water (3 mL), then the water was extracted with DCM (2 x 15 mL). The dried residue was washed with Hexane:Et_2O 1:1 (3 x 5 mL) then dried under reduced pressure to yield the title compound **156** as an oil (0.0954 g, 84 %, α:β = 1:1.4); **^{1}H NMR** δ_H (500 MHz, Acetonitrile-d_3) 8.45 (1 H, s, NCHN α), 8.40 (1 H, s, NCHN β), 7.41 – 7.18 (m, H_{arom}), 4.93 – 4.87 (m, H-1 α), 4.87 – 4.65 (m, PhCH_2), 4.59 – 4.47 (m, PhCH_2), 4.42 (1 H, d, *J* 7.8, H-1 β), 4.31 – 4.16 (m, CH_2), 3.84 – 3.60 (m), 3.71 (3 H, s, NCH_3 β), 3.63 (3 H, s, NCH_3 α), 3.55 – 3.48 (m), 3.43 (1 H, dt, *J* 10.3, 6.1, (C-1)OC*H*H α), 3.29 (1 H, t, *J* 8.5, H-2 β), 2.11 (4 H, dt, *J* 9.7, 6.0, $CH_2CH_2CH_2$); **^{13}C NMR** δ_C (126

MHz, Acetonitrile-d_3) 140.07, 139.87, 139.85, 139.54, 139.50, 139.48, 139.43, 139.35 (8 4° C_{arom}), 137.29 (NCHN β), 137.23 (NCHN α), 129.44, 129.37, 129.33, 129.27, 129.23, 129.03, 128.96, 128.90, 128.86, 128.81, 128.78, 128.66, 128.62, 128.58, 128.54, 128.47, 124.42, 124.31, 123.59, 123.48 (44 C_{arom}), 103.98 (C-1 β), 97.64 (C-1 α), 85.25, 83.01 (C-2 β), 82.58, 81.08, 78.91, 78.86, 76.03, 75.92, 75.66, 75.47, 75.21, 75.19, 73.83, 73.80, 73.61, 71.53, 69.98, 69.96, 66.57, 65.35 ((C-1)O*C*H_2), 48.14 (CH_2), 47.77 (CH_2), 36.70 (NCH_3), 36.65 (NCH_3), 30.72 ($CH_2CH_2CH_2$), 30.21 ($CH_2CH_2CH_2$); **m/z** (ESI-HRMS) $C_{41}H_{47}N_2O_6^+$ ([M – OTf]$^+$) calculated: 663.3429; found 663.3407; **IR** ν_{max}/cm^{-1} 3155w, 3032w, 2871w, 1726, 1603w, 1574w, 1497, 1454, 1361, 1258s, 1225, 1160, 1069s, 1030s, 915w, 827, 740, 699, 638, 623.

1-(4-(hydroxymethyl)benzyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate 157

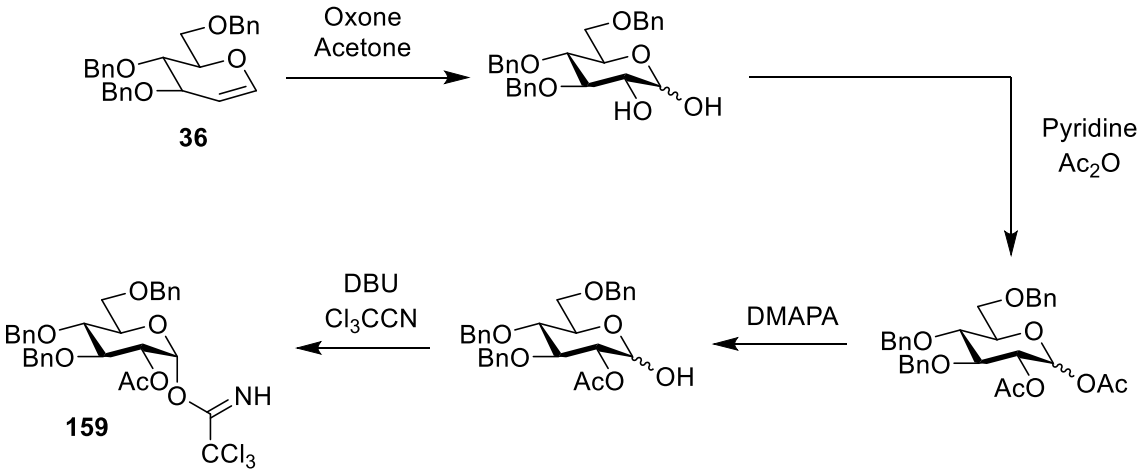


157

4-(Chloromethyl)benzyl alcohol (2.00 g mL, 12.77 mmol), 1-methylimidazole (4.07 mL, 51.08 mmol), potassium trifluoromethanesulfonate (4.69 g, 24.90 mmol) and anhydrous acetonitrile (50 mL) were added to an oven-dried flask. The resulting mixture was heated under reflux at 90 °C under a N_2 atmosphere for 21 h. ^{1}H NMR analysis of the crude material revealed that the alcohol starting material had been converted to the product quantitatively. The reaction mixture was filtered and the solvent was removed under reduced pressure. The crude product was triturated three times with hexane and three times using Et_2O:DCM 13:7. To remove final traces of 1-methylimidazole, a 0.930 g portion of the crude product was dissolved in a mixture of water:MeCN (95:5, 4.5 mL) to give a cloudy mixture, which was passed through a syringe-tip filter to remove the solids. The resulting solution was purified by reverse phase HPLC (Water:MeCN) and freeze-dried, to afford the title compound **157** (0.511 g, 55 %) as a colourless oil, with spectroscopic details in accordance with the literature;[173] **^{1}H NMR** δ_H (400 MHz, Acetonitrile-d_3) 8.56 (1 H, s, NCHN), 7.45 – 7.31 (6 H, m, H_{arom}), 5.30 (2 H, s, NCH_2), 4.59 (2 H, s, HOCH_2), 3.81 (3 H, s, CH_3); **^{13}C NMR** δ_C (101 MHz, Acetonitrile-d_3) 144.36 (4° $C_{arom}CH_2OH$), 137.10 (NCHN), 133.32 (4° $C_{arom}CH_2N$), 129.49, 128.24 (4 C_{arom}), 124.92, 123.15 (2 N*C*HCHN), 64.03 ($HOCH_2$), 53.48 (NCH_2), 36.87 (CH_3); **^{19}F NMR** δ_F (377 MHz,

Acetonitrile-d_3) -79.31 (CF_3); **m/z** (ESI-MS+) $C_{12}H_{15}N_2O^+$ ([M – OTf]$^+$) calculated: 203.1179; found 203.1188.

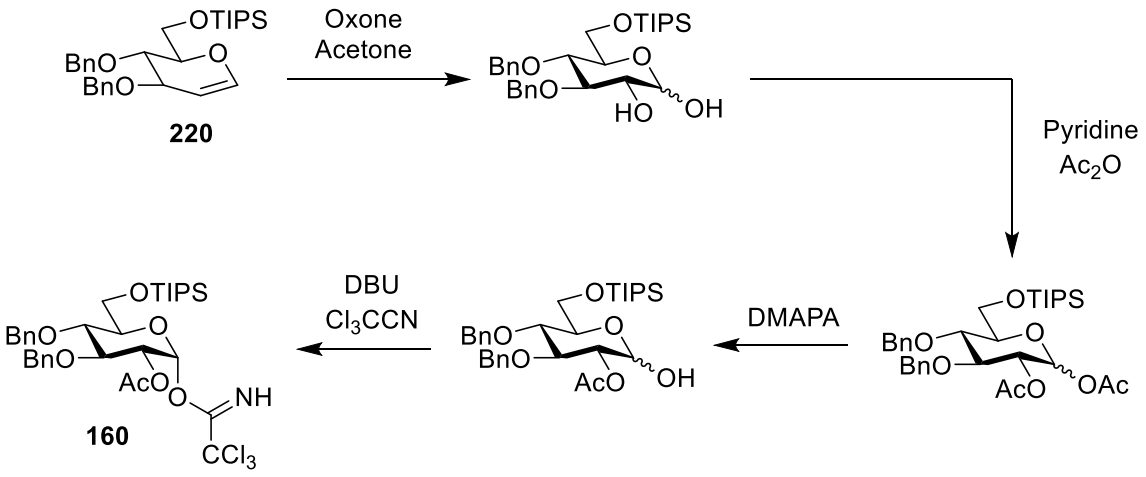
2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl trichloroacetimidate 159



Tri-*O*-benzyl-D-glucal **36** (2.00 g, 4.80 mmol) was dissolved in a 4:1 acetone:water mixture (40 mL) and stirred at RT. A solid mixture of Oxone® (18.44 g, 60.00 mmol) and sodium bicarbonate (10.08 g, 120.00 mmol) was added in small portions over 60 min with stirring to give a milky mixture. 40 min after all the Oxone®/sodium bicarbonate mixture had been added TLC (Hexane:EtOAc 6:4) showed the reaction to be complete. Most of the acetone solvent was removed under reduced pressure and EtOAc (100 mL) and water (100 mL) were added to the residue. Product was extracted into the organic phase, and the aqueous phase was washed with further EtOAc (2 x 100 mL), then all EtOAc washings were combined. The resulting organic solution was washed with water (100 mL) and brine (100 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (Hexane:EtOAc 7:3 → 6:4 → 1:1) to give the diol intermediate as an anomeric mixture. This intermediate was dissolved in anhydrous pyridine (32 mL) and acetic anhydride (16 mL) and stirred at RT for 16 h under a nitrogen atmosphere, after which time TLC (Hexane:EtOAc 6:4) showed the reaction to be complete. The reaction mixture was diluted with DCM (100 mL) and washed with 2 M HCl $_{(aq.)}$ (3 x 40 mL), 5 M HCl $_{(aq.)}$ (40 mL), $NaHCO_3$ $_{(sat.\ aq.)}$ (2 x 40 mL) and brine (40 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. The resulting acetylated intermediate was dried under vacuum for 1 h before being dissolved in anhydrous

THF (20 mL) under a nitrogen atmosphere. DMAPA (3.03 mL, 24.00 mmol) was added and the resulting solution was stirred at RT for 90 min, after which time TLC (Hexane:EtOAc 6:4) showed the reaction to be complete. The reaction mixture was diluted with DCM (100 mL), washed with 1 M HCl $_{(aq.)}$ (70 mL) and brine (70 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure to give the free hemiacetal intermediate as an anomeric mixture. This intermediate was dried under vacuum for 1 h before being dissolved in anhydrous DCM (30 mL). Trichloroacetonitrile (7.22 mL, 72.00 mmol) and DBU (0.36mL, 2.40 mmol) were added and the resulting solution was stirred at RT for 70 min under a nitrogen atmosphere, after which time TLC (Hexane:EtOAc 6:4) showed the reaction to be complete. Solvent was then removed under reduced pressure and the crude product was purified by column chromatography (Hexane:EtOAc 95:5 → 9:1) using neutralised silica (1 % NEt_3) to give the title compound **159** as an oil (0.78 g, 26 % over 4 steps) with spectroscopic details in accordance with the literature;[174-176] **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 8.57 (1 H, s, NH), 7.36 – 7.27 (13 H, m, H_{arom}), 7.21 – 7.16 (2 H, m, H_{arom}), 6.53 (1 H, d, *J* 3.6, H-1), 5.08 (1 H, dd, *J* 10.0, 3.6, H-2), 4.86 (2 H, app t, *J* 11.0, PhCH_2), 4.78 (1 H, d, *J* 11.4, PhCH*H*), 4.64 (1 H, d, *J* 12.0, PhC*H*H), 4.59 (1 H, d, *J* 10.6, PhCH*H*), 4.51 (1 H, d, *J* 12.0, PhCH*H*), 4.10 (1 H, t, *J* 9.6, H-3), 4.02 (1 H, ddd, *J* 10.1, 3.4, 1.9, H-5), 3.89 (1 H, dd, *J* 10.1, 9.2, H-4), 3.82 (1 H, dd, *J* 11.1, 3.4, H-6a), 3.71 (1 H, dd, *J* 11.1, 1.9, H-6b), 1.94 (3 H, s, CH_3); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) 170.17 (C=O), 161.15 (CNH), 138.41, 137.98, 137.95 (3 4° C_{arom}), 128.60, 128.54, 128.53, 128.28, 128.08, 128.03, 127.93, 127.87 (C_{arom}), 94.18 (C-1), 91.19 (CCl_3), 79.63 (C-3), 77.16 (C-4), 75.56 (Ph*C*H_2), 75.53 (Ph*C*H_2), 73.65, 73.58 (Ph*C*H_2, C-5), 72.52 (C-2), 68.02 (C-6), 20.73 (CH_3); **m/z** (ESI-MS+) $C_{31}H_{32}Cl_3NO_7Na^+$ ($[M + Na]^+$) calculated 658.1; found 658.1; $C_{29}H_{31}O_6^+$ ($[M – OC(NH)CCl_3]^+$) calculated 475.2; found 475.2.

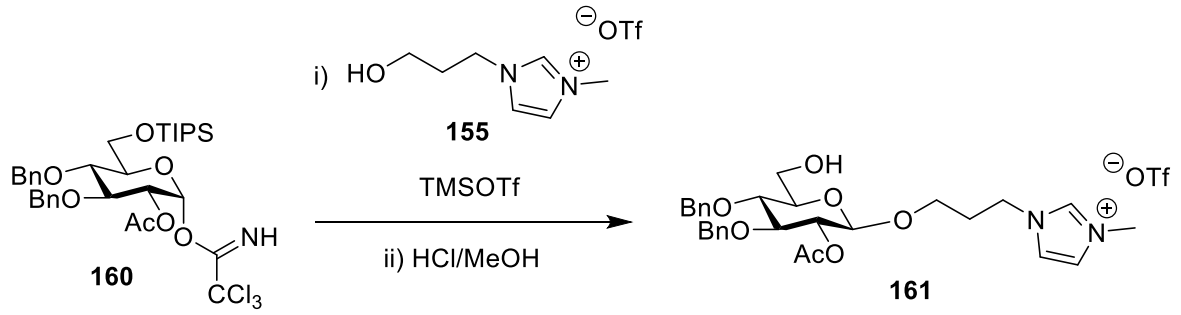
2-*O*-Acetyl-3,4-di-*O*-benzyl-6-*O*-triisopropylsilyl-α-D-glucopyranosyl trichloroacetimidate 160



3,4-Di-*O*-benzyl-6-*O*-(triisopropylsilyl)-D-glucal **220** (20.47 g, 42.40 mmol) was dissolved in a 4:1 acetone:water mixture (355 mL) and stirred at RT. A solid mixture of Oxone® (32.59 g, 106.01 mmol) and sodium bicarbonate (17.81 g, 212.02 mmol) was added in small portions over 90 min with stirring to give a milky mixture. 1 h after all the Oxone®/sodium bicarbonate mixture had been added, TLC (Hexane:EtOAc 7:3) showed the reaction to be complete. Most acetone solvent was removed under reduced pressure and EtOAc (100 mL) and water (100 mL) were added to the residue. Product was extracted into the organic phase, and the aqueous phase was washed with further EtOAc (2 x 100 mL), then all EtOAc washings were combined. The resulting organic solution was washed with water (100 mL) and brine (100 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. The crude product was purified by normal phase HPLC (Hexane:EtOAc) to give the diol intermediate as an anomeric mixture (11.80 g, 22.84 mmol). This intermediate was dissolved in anhydrous pyridine (184 mL) and acetic anhydride (46 mL) and stirred at RT for 20 h under a nitrogen atmosphere, after which time TLC (Hexane:EtOAc 8:2) showed the reaction to be complete. Most of the solvent was removed under reduced pressure, then the residue was diluted with DCM (200 mL) and washed with 1 M $HCl_{(aq.)}$ (2 x 100 mL), $NaHCO_{3\ (sat.\ aq.)}$ (100 mL) and brine (100 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. The resulting acetylated intermediate was dried under vacuum for 1 h before being dissolved in anhydrous THF (120 mL) under a

nitrogen atmosphere. DMAPA (14.41 mL, 114.20 mmol) was added and the resulting solution stirred at RT for 2.5 h, after which time TLC (Hexane:EtOAc 8:2) showed the reaction to be complete. Solvent was removed under reduced pressure and the residue was dissolved in DCM (200 mL) and washed with 1 M $HCl_{(aq.)}$ (200 mL) and brine (200 mL). As a result of very poor phase separation during workup, $NaHCO_{3\ (sat.\ aq.)}$ was added to aid phase separation. The DCM phase was dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. Following purification by normal phase HPLC (Petroleum Ether b.p. 40-60 ˚C:EtOAc) the free hemiacetal intermediate was obtained as an anomeric mixture (5.63 g, 10.08 mmol). This intermediate was dried under vacuum for 1 h before being dissolved in anhydrous DCM (216 mL) and cooled to 5 ˚C. Trichloroacetonitrile (10.10 mL, 100.75 mmol) and DBU (0.60 mL, 4.03 mmol) were added and the resulting solution was stirred at RT for 40 min under a nitrogen atmosphere, after which time TLC (Hexane:EtOAc 9:1) showed the reaction to be complete. Solvent was then removed under reduced pressure and the crude product was purified by column chromatography (Hexane:EtOAc 93:7) to give the title compound **160** as an oil (4.95 g, 17 % over 4 steps); **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 8.52 (1 H, s, NH), 7.38 – 7.26 (10 H, m, H_{arom}), 6.51 (1 H, d, *J* 3.6, H-1), 5.01 (1 H, dd, *J* 10.0, 3.6, H-2), 4.89 (2 H, app t, *J* 10.9, PhCH_2), 4.77 (2 H, app dd, *J* 11.0, 2.7, PhCH_2), 4.11 (1 H, ddd, *J* 9.7, 7.3, 1.7, H-3), 4.03 (1 H, dd, *J* 11.4, 2.6, H-6a), 3.95 (1 H, d, *J* 11.1, H-6b), 3.91 – 3.86 (2 H, m, H-4, H-5), 1.93 (3 H, s, C(O)CH_3), 1.12 – 1.03 (21 H, m, TIPS); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) 170.22 (C=O), 161.17 (CNH), 138.47, 138.23 (2 4˚ C_{arom}), 128.65, 128.54, 128.25, 128.06, 128.04, 127.89 (C_{arom}), 94.29 (C-1), 91.33 (CCl_3), 79.67 (C-3), 77.02 (C-4), 75.63 (Ph*C*H_2), 75.55 (Ph*C*H_2), 74.90 (C-5), 72.71 (C-2), 62.03 (C-6), 20.76 (C(O)*C*H_3), 18.16, 18.12, 12.12 (TIPS); **m/z** (ESI-HRMS) $C_{33}H_{46}Cl_3NO_7SiNa^+$ ([M + Na]$^+$) calculated 724.2001; found 724.1983; $C_{31}H_{45}O_6Si^+$ ([M – OC(NH)CCl_3]$^+$) calculated 541.2980; found 541.2968; **IR** ν_{max}/cm^{-1} 3345w, 3031w, 2942, 2893, 2866, 1749 (C=O), 1673 (C=N), 1497, 1455, 1365, 1294, 1228, 1157, 1136, 1060s, 1049s, 1028, 1014, 969, 914, 883, 831, 794, 736, 697, 682, 645; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{23}}$ + 53 [*c* 1.22, DCM].

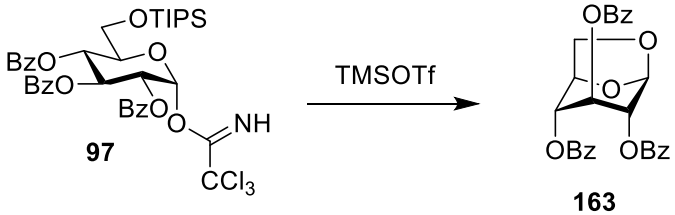
3-(3-Methylimidazolium)-1-propyl 2-*O*-acetyl-3,4-di-*O*-benzyl-α-D-glucopyranoside trifluoromethanesulfonate 161



Following the general procedure for I-Tag glycosylations in flow using glycosyl acceptor 1-(3-hydroxypropyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate **155** (0.0232 g, 0.080 mmol, 1 eq) and glycosyl donor 2-*O*-acetyl-3,4-di-*O*-benzyl-6-*O*-triisopropylsilyl-α-D-glucopyranosyl trichloroacetimidate **160** (0.1125 g, 0.160 mmol, 2 eq). 0.40 mL of anhydrous acetonitrile was added to the donor/acceptor vial, resulting in a solution of volume 0.50 mL and therefore approximately 0.160 M in acceptor and 0.320 M in donor. To the other vial, 0.80 mL of anhydrous acetonitrile was added, followed by trimethylsilyl trifluoromethanesulfonate (8.7 µL, 0.048 mmol) to make a 0.06 M solution. The flow reaction was performed at RT. Reaction solution was collected into a mixture of DCM and sodium acetate for a total of 6 min. Following completion of the flow reaction, the product mixture was filtered and the solvent was removed under reduced pressure. The crude glycosylation product was washed with hexane (2 x 3 mL) and hexane:Et_2O 3:1 (2 x 3 mL). The residue was dissolved in DCM (2 mL) and HCl, 0.8 M in MeOH (3 mL) and the resulting solution was stirred at RT for 1 h in air to fully remove the silyl ether group from the product. Solvent was then removed under reduced pressure and the residue was washed with water (2 x 3 mL). The combined aqueous washes were washed with DCM (4 x 6 mL) and the combined DCM washes were added back to the flask containing product residue. The resulting solution was dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. The residue was washed with hexane:Et_2O 1:1 (3 x 3 mL) then dried under reduced pressure to yield the title compound **161** (0.0060 g, 14 % over 2 steps); **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 9.10 (1 H, s, NCHN), 7.36 – 7.20 (12 H, m, H_{arom}), 4.80 (2 H, app dd, *J* 11.1, 5.3, PhCH_2), 4.70 – 4.62 (3 H, m, PhCH_2, H-2), 4.35 – 4.25 (3 H, m, H-1, NCH_2),

3.98 – 3.95 (1 H, m, H-6a), 3.94 (3 H, s, NCH_3), 3.91 – 3.83 (1 H, m, (C-1)OC*H*H), 3.74 (1 H, dd, *J* 12.1, 4.6, H-6b), 3.68 – 3.62 (2 H, m, H-3, (C-1)OCH*H*), 3.60 (1 H, t, *J* 9.1, H-4), 3.38 (1 H, ddd, *J* 9.2, 4.6, 2.3, H-5), 2.12 – 2.05 (2 H, m, NCH_2CH_2), 1.92 (3 H, s, (C=O)CH_3); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) 169.93 (C=O), 138.20, 137.93 (2 4˚ C_{arom}), 137.61 (NCHN), 128.66, 128.59, 128.23, 128.12, 127.98, 127.96 (C_{arom}), 123.30 (N*C*HCHN), 122.65 (NCH*C*HN), 100.59 (C-1), 82.64 (C-3), 77.85 (C-4), 75.80 (C-5), 75.37, 75.14 (2 PhCH_2), 73.19 (C-2), 66.15 ((C-1)OCH_2), 61.03 (C-6), 47.82 (NCH_2), 36.45 (NCH_3), 30.03 (NCH_2CH_2), 21.04 ((C=O)CH_3); **m/z** (ESI-HRMS) $C_{29}H_{37}N_2O_7^+$ ([M – OTf]$^+$) calculated: 525.2595; found 525.2591; (TLC-MS- (ESI)) $CF_3O_3S^-$ ([OTf]$^-$) calculated 148.9; found 149.1; **IR** ν_{max}/cm^{-1} 3475br (OH), 3153w, 3115w, 2959, 2924, 2856, 1743 (C=O), 1574, 1497w, 1455, 1430w, 1374, 1255s, 1226, 1160, 1076, 1046, 1030s, 912w, 804w, 755, 701, 639s, 624, 573, 518, 472, 418w; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{24}}$ 0 [*c* 0.41, DCM]. Note that due to sparing solubility of the product in DCM, the solution used to determine optical rotation was passed through a syringe tip filter prior to the measurement being taken.

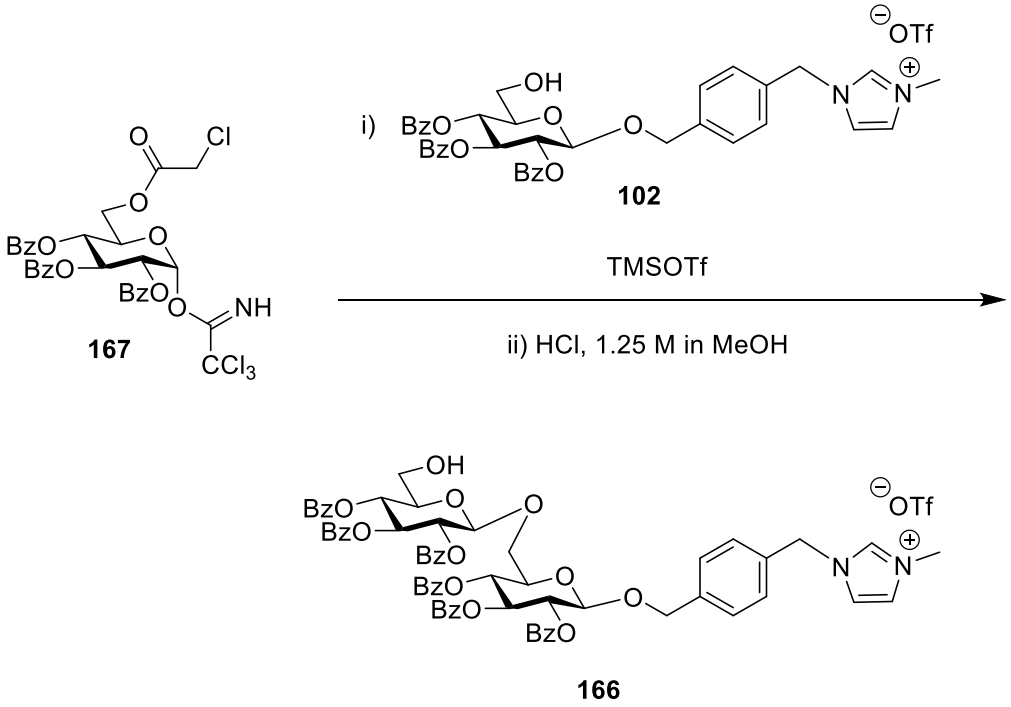
1,6-anhydro-β-D-glucose-2,3,4-tri-*O*-benzoate 163



2,3,4-Tri-*O*-benzoyl-6-*O*-triisopropylsilyl-α-D-glucopyranosyl trichloroacetimidate **97** (0.1269 g, 0.160 mmol, 1.0 eq) was dried under vacuum. 0.4 mL of anhydrous acetonitrile was added, followed by trimethylsilyl trifluoromethanesulfonate (8.7 μL, 0.048 mmol). The resulting solution was swirled under nitrogen for 3 minutes, then reagent grade acetonitrile was added with air. The solvent was removed under reduced pressure and the resultant solid was washed with acetonitrile (3 x 0.7 mL). The residue was dried under vacuum to give the title compound **163** as a white solid with spectroscopic details in accordance with the literature;[160] **^{1}H NMR** δ_H (400 MHz, Chloroform-*d*) 8.19 – 8.11 (4 H, m, H_{arom}), 8.11 – 8.06 (2 H, m, H_{arom}), 7.66 – 7.56 (3 H, m, H_{arom}), 7.53 – 7.39 (6 H, m, H_{arom}), 5.75 (1 H, bs, H-1), 5.45 (1 H, app p, *J* 1.7, H-2,3 or 4), 5.12 – 5.09 (1 H, m, H-2,3 or 4), 5.09 – 5.06 (1 H, m, H-2,3 or 4), 4.90 (1 H, dq, *J* 4.2, 1.4, H-5), 4.39 (1 H, dd, *J* 7.8, 1.0, H-6a), 3.98 (1 H, dd, *J* 7.7, 5.7, H-6b); **^{13}C NMR** δ_C (101 MHz, Chloroform-*d*) 165.48, 165.23, 164.82 (3 C=O), 133.82, 133.71, 133.67,

130.18, 130.15, 130.05 (C_{arom}), 129.46, 129.44, 129.19 (3 4° C_{arom}), 128.77, 128.58 (C_{arom}), 99.60 (C-1), 73.96 (C-5), 70.31, 69.92, 69.22 (C-2,3,4), 65.66 (C-6); **m/z** (ESI-MS+) $C_{27}H_{22}O_8Na^+$ ($[M + Na]^+$) calculated 497.1; found 497.1; $C_{27}H_{23}O_8^+$ ($[M + H]^+$) calculated 475.1; found 475.1.

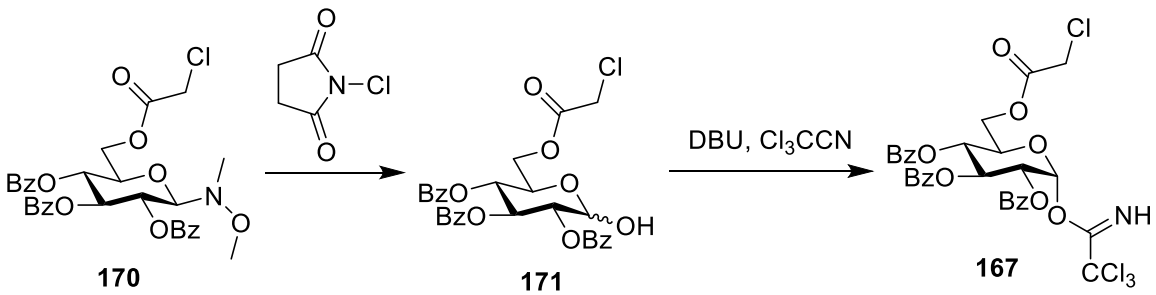
4-(1-Methyl-3-methyleneimidazolium)-benzyl 2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl)-β-D-glucopyranoside trifluoromethanesulfonate 166



Following the general procedure for I-Tag glycosylations in flow using glycosyl acceptor 4-(1-methyl-3-methyleneimidazolium)benzyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside trifluoromethanesulfonate **102** (0.3510 g, 0.425 mmol, 1 eq) and glycosyl donor 2,3,4-tri-*O*-benzoyl-6-*O*-chloroacetyl-α-D-glucopyranosyl trichloroacetimidate **167** (0.6057 g, 0.849 mmol, 2.0 eq). 2.12 mL of anhydrous acetonitrile was added to the donor/acceptor vial, resulting in a solution of volume 2.6 mL and therefore approximately 0.163 M in acceptor and 0.327 M in donor. To the other vial, 3.5 mL of anhydrous acetonitrile was added, followed by trimethylsilyl trifluoromethanesulfonate (38.0 μL, 0.210 mmol) to make a 0.06 M solution. The flow reactor was submerged in an oil bath at 50 °C to allow the flow reaction to occur at 50 °C. Reaction solution was collected for a total of 39 min. The product solution from the flow reactor was collected directly into a mixed solution of DCM (6.8 mL) and HCl, 1.25 M in MeOH (6.8 mL, 8.49 mmol) to begin immediate chloroacetate deprotection. The reaction solution was stirred at RT in air for 16 h, after which time TLC-MS

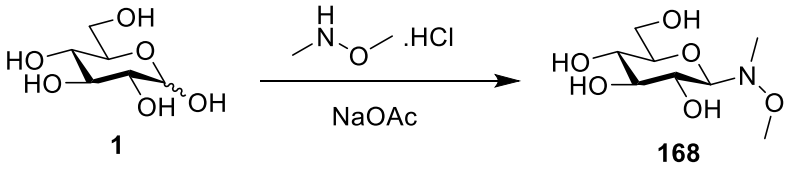
showed reaction to be complete. The reaction mixture was diluted with DCM and then washed with water. The aqueous phase was washed with a further portion DCM, then the DCM phases were combined, dried with magnesium sulfate, filtered and the solvent was removed under reduced pressure. The dried residue was washed with Et_2O three times, DCM:Et_2O 1:9 twice and DCM:Et_2O 86:14 once then dried under reduced pressure. Desired product **166** and unreacted glycosyl acceptor **102** were separated by normal-phase HPLC (DCM:MeOH) to yield the title compound **166** as a single anomer (0.0822 g, 15 %) with spectroscopic details in accordance with the literature;[146] **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 9.14 (1 H, s, NCHN), 7.94 – 7.85 (8 H, m, H_{arom}), 7.80 – 7.77 (2 H, m, H_{arom}), 7.77 – 7.73 (2 H, m, H_{arom}), 7.55 – 7.22 (19 H, m, H_{arom}, NC*H*CHN), 7.22 – 7.19 (2 H, m, H_{arom}), 7.18 (1 H, t, *J* 1.9, NCHC*H*N), 7.16 – 7.13 (2 H, m, H_{arom}), 5.88 (1 H, t, *J* 9.7, H-3'), 5.79 (1 H, t, *J* 9.6, H-3), 5.45 – 5.40 (3 H, m, H-2', H-2, H-4), 5.38 (1 H, t, *J* 9.7, H-4'), 5.29 (2 H, d, *J* 1.8, NCH_2), 4.91 (1 H, d, *J* 7.9, H-1'), 4.72 (1 H, d, *J* 7.9, H-1), 4.62 (1 H, d, *J* 12.6, (C-1)OC*H*H), 4.42 (1 H, d, *J* 12.6, (C-1)OCH*H*), 4.16 (1 H, dd, *J* 10.9, 2.7, H-6a), 4.00 (1 H, ddd, *J* 9.4, 6.2, 2.7, H-5), 3.91 (3 H, s, NCH_3), 3.85 (1 H, dd, *J* 11.0, 6.3, H-6b), 3.81 – 3.73 (2 H, m, H-5', H-6a'), 3.65 – 3.60 (1 H, m, H-6b'); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) 166.12, 165.90, 165.80, 165.71, 165.36, 165.27 (6 C=O), 138.44 (4° C_{arom}), 137.06 (NCHN), 133.84, 133.74, 133.59, 133.49, 133.43, 133.36 (C_{arom}), 132.31 (4° C_{arom}), 130.01, 130.00, 129.90, 129.84, 129.82, 129.78, 129.28, 129.20, 129.16, 129.04, 128.93, 128.89, 128.82, 128.73, 128.65, 128.57, 128.45, 128.39 (C_{arom}), 123.73 (N*C*HCHN), 122.07 (NCH*C*HN), 101.08 (C-1'), 99.78 (C-1), 74.79 (C-5'), 73.49 (C-5), 72.91 (C-3', C-3), 71.94, 71.90 (C-2', C-2), 70.09 (C-4), 69.41 (C-4'), 68.37 (C-6), 61.10 (C-6'), 53.27 (NCH_2), 36.60 (NCH_3); **m/z** (TLC-MS+ (ESI)) $C_{66}H_{59}N_2O_{17}^+$ ([M - OTf]$^+$) calculated 1151.4; found 1151.4.

2,3,4-Tri-*O*-benzoyl-6-*O*-chloroacetyl-α-D-glucopyranosyl trichloroacetimidate 167



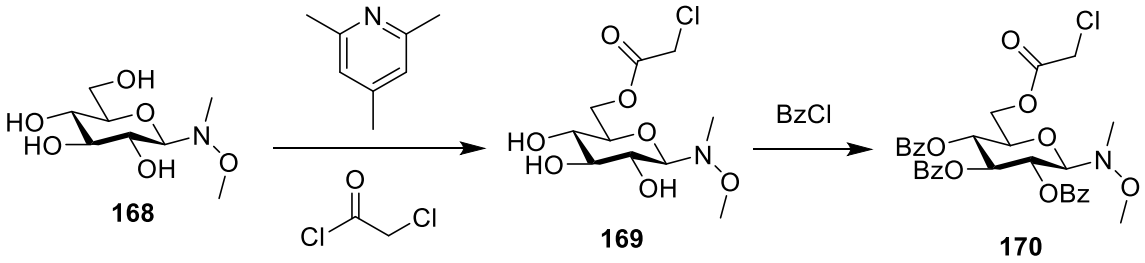
N,O-Dimethyl-*N*-(2,3,4-tri-*O*-benzoyl-6-*O*-chloroacetyl-β-D-glucopyranosyl)hydroxylamine **170** (8.78 g, 14.35 mmol) was dissolved in THF:Water 9:1 (528 mL) and *N*-chlorosuccinimide (3.83 g, 28.69 mmol) was added. The resulting solution was warmed to 60 ˚C and stirred for 2 h 40, after which time TLC (Hexane:EtOAc 6:4) showed the reaction to be complete. The solution was cooled to RT, then the solvent was removed under reduced pressure. The crude residue was purified by column chromatography (Hexane:EtOAc 3:1 → 7:3) to give the free hemiacetal intermediate **171** as an anomeric mixture (7.11 g), which was used directly in the next step. Hemiacetal intermediate **171** was dried under vacuum for 1 h before being dissolved in anhydrous DCM (250 mL). The resulting solution was cooled to 5 ˚C and trichloroacetonitrile (12.53 mL, 124.97 mmol) and DBU (0.75 mL, 5.00 mmol) were added. The resulting solution was stirred for 1 h under a nitrogen atmosphere, after which time TLC (Hexane:EtOAc 7:3) showed the reaction to be complete. Water was added and the two phases were washed together. The DCM phase was then separated, dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. Diethyl ether was added to the residue, and the resulting solution was decanted off, leaving a dark coloured impurity behind. The solvent was then removed under reduced pressure and the crude product was purified by column chromatography (Hexane:EtOAc 85:15 → 8:2) using neutralised silica (1 % NEt_3) to give the title compound **167** as a solid (2.55 g, 25 % over 2 steps); **^{1}H NMR** δ_H (400 MHz, Chloroform-*d*) 8.67 (1 H, s, NH), 7.98 – 7.92 (4 H, m, H_{arom}), 7.88 – 7.83 (2 H, m, H_{arom}), 7.56 – 7.48 (2 H, m, H_{arom}), 7.47 – 7.33 (5 H, m, H_{arom}), 7.33 – 7.27 (2 H, m, H_{arom}), 6.81 (1 H, d, *J* 3.7, H-1), 6.25 (1 H, t, *J* 10.0, H-3), 5.71 (1 H, t, *J* 10.0, H-4), 5.59 (1 H, dd, *J* 10.2, 3.7, H-2), 4.52 (1 H, ddd, *J* 10.3, 4.3, 2.7, H-5), 4.46 – 4.36 (2 H, m, H-6a, H-6b), 4.12 (2 H, d, *J* 3.2, CH_2Cl); **^{13}C NMR** δ_C (101 MHz, Chloroform-*d*) 167.12 (O*C*(O)CH_2Cl), 165.73, 165.52, 165.44 (3 C=O (Bz)), 160.66 (CNH), 133.88, 133.75, 133.50, 130.07, 130.06, 129.84, 128.92, 128.67, 128.63, 128.60, 128.56, 128.51 (C_{arom}), 93.19 (C-1), 90.80 (CCl_3), 70.72 (C-2), 70.47 (C-5), 70.07 (C-3), 68.43 (C-4), 63.53 (C-6), 40.78 (CH_2Cl); **m/z** (ESI-HRMS) $C_{31}H_{25}Cl_4O_{10}NNa^+$ ([M + Na]$^+$) calculated 734.0125; found 734.0113; **IR** ν_{max}/cm^{-1} 1766, 1731s (C=O), 1677, 1601, 1584, 1451, 1314, 1264s, 1177, 1143, 1106, 1094, 1069, 1024, 970, 918, 836, 795, 708s, 686, 645; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ + 49 [*c* 1.68, DCM].

***N,O*-Dimethyl-*N*-(β-D-glucopyranosyl)hydroxylamine 168**



D-Glucose **1** (10.00 g, 55.51 mmol) was dissolved in water (75 mL) and cooled to 0 ℃. *N,O*-Dimethylhydroxylamine hydrochloride (5.96 g, 61.06 mmol) and sodium acetate (5.01 g, 61.06 mmol) were dissolved in a minimal volume of water and the resulting solution was added to the glucose solution dropwise. The resulting reactant solution was stirred at RT in air for 21 h, after which time TLC (DCM:MeOH 7:3) showed formation of product, but still significant amounts of starting material remaining. A further portion of *N,O*-dimethylhydroxylamine hydrochloride (2.71 g, 27.75 mmol) and sodium acetate (2.28 g, 27.75 mmol) were dissolved in a minimal volume of water and the resulting solution was added to the glucose solution to encourage more complete reaction. The resulting reactant solution was stirred at RT in air for a further 20 h, then solvent was evaporated. To the crude product, methanol (50 mL) was added and the mixture was sonicated. The mixture was then filtered with additional methanol washing and the filtrate was collected, then the solvent was removed under reduced pressure. Following column chromatography (DCM:MeOH 85:15), the title compound **168** was obtained as a white solid (7.64 g, 62 %) with spectroscopic details in accordance with the literature;[177] **^{1}H NMR** δ_H (400 MHz, Deuterium Oxide) 4.17 – 4.08 (1 H, m, H-1), 3.94 (1 H, dd, *J* 12.4, 2.0, H-6a), 3.79 – 3.72 (1 H, m, H-6b), 3.60 (3 H, s, OCH_3), 3.58 – 3.49 (2 H, m, H-2, H-4), 3.47 – 3.34 (2 H, m, H-3, H-5), 2.77 (3 H, s, NCH_3); **^{13}C NMR** δ_C (101 MHz, Deuterium Oxide) 95.20 (C-1), 79.72 (C-5), 79.43 (C-2), 72.12 (C-4), 71.84 (C-3), 63.24 (C-6), 62.28 (OCH_3), 40.35 (NCH_3); **m/z** (ESI-MS+) $C_8H_{17}NO_6Na^+$ ([M + Na]$^+$) calculated 246.0948; found 246.0957.

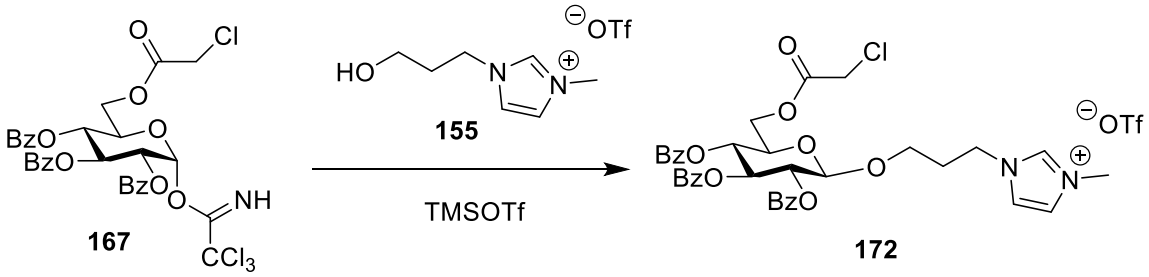
***N,O*-Dimethyl-*N*-(2,3,4-tri-*O*-benzoyl-6-*O*-chloroacetyl-β-D-glucopyranosyl)hydroxylamine 170**



Following a modification of the procedure reported by Glaudemans and co-workers,[178] *N,O*-dimethyl-*N*-(β-D-glucopyranosyl)hydroxylamine **168** (7.42 g, 33.24 mmol) was dried under vacuum for 1 h. Anhydrous DMF (167 mL) was added under a nitrogen atmosphere and the resulting solution was cooled to -60 ˚C using a chloroform/dry ice bath. 2,4,6-Collidine (5.84 mL, 44.21 mmol) was then added. In a separate dry flask, chloroacetyl chloride (3.44 mL, 43.21 mmol) was dissolved in anhydrous toluene (11 mL) and the resulting solution was added to the sugar solution dropwise. The reacting solution was stirred for 30 min at -60 ˚C under nitrogen, after which time TLC (DCM:MeOH 9:1) showed the reaction to be complete. The solution was warmed to RT, toluene (166 mL) was added and the mixture was filtered to remove solids. Solvent was then removed under reduced pressure. To the residue, acetone (55 mL) was added and the resulting mixture was sonicated for 5 min. The mixture was then filtered, solids were discarded, and solvent was removed from the filtrate under reduced pressure. The crude residue was purified by normal phase HPLC (DCM:MeOH) to give *N,O*-dimethyl-*N*-(6-*O*-chloroacetyl-β-D-glucopyranosyl)hydroxylamine **169** (7.42 g), a portion of which (4.36 g) was used directly in the next step. *N,O*-Dimethyl-*N*-(6-*O*-chloroacetyl-β-D-glucopyranosyl)hydroxylamine **169** (4.36 g) was dried under vacuum for 1 h. Anhydrous acetonitrile (29 mL) was added under a nitrogen atmosphere and the resulting mixture was heated to 45 ˚C. In a separate dry flask, benzoyl chloride (9.13 mL, 78.56 mmol) and anhydrous pyridine (5.88 mL, 72.74 mmol) were dissolved in anhydrous acetonitrile (29 mL) and the resulting solution was quickly added to the sugar suspension. The reacting solution was stirred for 1 h 15 min at 45 ˚C under nitrogen, after which time TLC (Hexane:EtOAc 8:2) showed the reaction to be complete. The reaction solution was cooled to RT, then EtOAc (100 mL) and water (100 mL) were added. The EtOAc phase was washed with

1 M HCl $_{(aq.)}$ (2 x 75 mL), $NaHCO_3$ $_{(sat.\ aq.)}$ (75 mL) and water (75 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. The crude material was purified by normal phase HPLC (Hexane:EtOAc) to give the title compound **170** as a white solid (5.45 g, 46 % over 2 steps); **^{1}H NMR** δ_H (400 MHz, Chloroform-*d*) 7.97 – 7.88 (4 H, m, H_{arom}), 7.84 – 7.79 (2 H, m, H_{arom}), 7.51 (2 H, ddt, *J* 7.9, 7.0, 1.3, H_{arom}), 7.45 – 7.34 (5 H, m, H_{arom}), 7.32 – 7.23 (2 H, m, H_{arom}), 5.91 (1 H, t, *J* 9.6, H-3), 5.65 (1 H, t, *J* 9.5, H-2), 5.55 (1 H, t, *J* 9.8, H-4), 4.58 (1 H, d, *J* 9.3, H-1), 4.47 (1 H, dd, *J* 12.2, 5.1, H-6a), 4.41 (1 H, dd, *J* 12.1, 3.1, H-6b), 4.06 (2 H, s, CH_2Cl), 4.02 (1 H, ddd, *J* 9.9, 5.0, 3.1, H-5), 3.43 (3 H, s, OCH_3), 2.79 (3 H, s, NCH_3); **^{13}C NMR** δ_C (101 MHz, Chloroform-*d*) 167.18 (O*C*(O)CH_2Cl), 165.90, 165.43, 165.36 (3 C=O (Bz)), 133.70, 133.36, 129.95, 129.88, 129.86, 129.55, 128.97, 128.82, 128.62, 128.50, 128.41 (C_{arom}), 92.68 (C-1), 74.06 (C-3), 73.62 (C-5), 69.55 (C-4), 69.10 (C-2), 64.34 (C-6), 60.48 (OCH_3), 40.83 (CH_2Cl), 37.59 (NCH_3); **m/z** (ESI-HRMS) $C_{31}H_{31}ClNO_{10}^+$ ([M + H]$^+$) calculated 612.1631; found 612.1630; **IR** ν_{max}/cm^{-1} 2941w, 1764, 1728s (C=O), 1601, 1451, 1411w, 1369w, 1314, 1280, 1260s, 1178, 1138, 1093, 1069, 1026, 1013, 975, 940w, 802w, 709, 687; $[\alpha]_D^{25}$ + 19 [*c* 2.16, DCM].

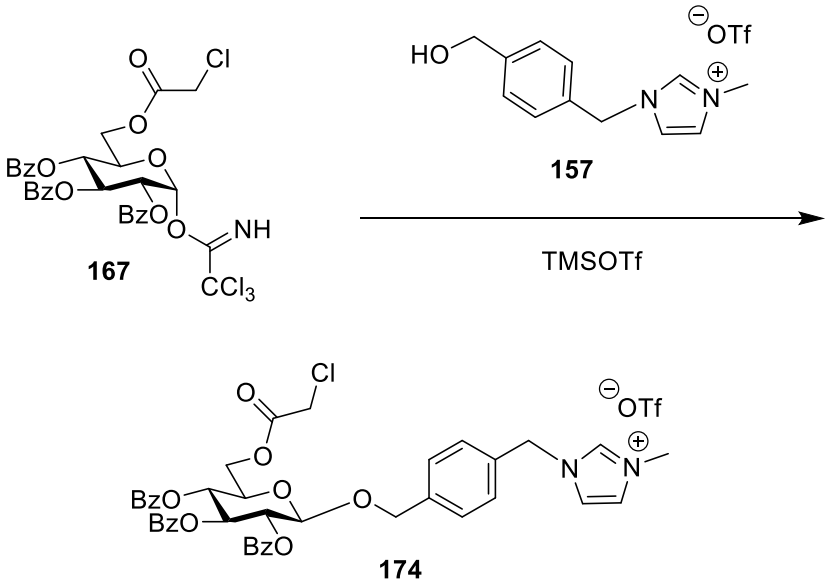
3-(3-Methylimidazolium)-1-propyl 2,3,4-tri-*O*-benzoyl-6-*O*-chloroacetyl-β-D-glucopyranoside trifluoromethanesulfonate 172



Following the general procedure for I-Tag glycosylations in flow using glycosyl acceptor 1-(3-hydroxypropyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate **155** (0.1161 g, 0.400 mmol, 1 eq) and glycosyl donor 2,3,4-tri-*O*-benzoyl-6-*O*-chloroacetyl-α-D-glucopyranosyl trichloroacetimidate **167** (0.5707 g, 0.800 mmol, 2.0 eq). 2.00 mL of anhydrous acetonitrile was added to the donor/acceptor vial, resulting in a solution of volume 2.4 mL and therefore approximately 0.167 M in acceptor and 0.333 M in donor. To the other vial, 3.00 mL of anhydrous acetonitrile was added, followed by trimethylsilyl

trifluoromethanesulfonate (32.6 μL, 0.180 mmol) to make a 0.06 M solution. The flow reactor was submerged in an ice-water bath to allow the flow reaction to occur at 0 ˚C. Reaction solution was collected for a total of 33 min. The crude product was dissolved in DCM (20 mL) and washed with water (4 mL), then the water was extracted with a further portion of DCM (20 mL). The dried residue was washed with Et_2O twice, DCM:Et_2O 5:95 three times and DCM:Et_2O 1:9 five times then dried under reduced pressure to yield the title compound **172** as a single anomer (0.2026 g, 67 %); **^{1}H NMR** δ_H (400 MHz, Acetonitrile-d_3) 8.31 (1 H, s, NCHN), 7.96 – 7.88 (4 H, m, H_{arom}), 7.78 – 7.74 (2 H, m, H_{arom}), 7.60 (2 H, m, H_{arom}), 7.52 (1 H, ddt, *J* 7.6, 6.7, 1.3, H_{arom}), 7.45 (4 H, td, *J* 7.7, 3.4, H_{arom}), 7.39 – 7.33 (2 H, m, H_{arom}), 7.28 (1 H, t, *J* 1.7, NC*H*CHN), 7.26 (1 H, t, *J* 1.7, (NCHC*H*N), 5.89 (1 H, t, *J* 9.6, H-3), 5.57 (1 H, t, *J* 9.7, H-4), 5.39 (1 H, dd, *J* 9.7, 8.0, H-2), 4.97 (1 H, d, *J* 8.0, H-1), 4.46 (1 H, dd, *J* 12.3, 4.9, H-6a), 4.40 (1 H, dd, *J* 12.4, 2.6, H-6b), 4.21 (2 H, s, CH_2Cl), 4.23 – 4.18 (1 H, m, H-5), 4.12 (2 H, td, *J* 6.8, 3.5, $OCH_2CH_2CH_2N$), 3.87 (1 H, ddd, *J* 11.0, 7.1, 4.8, O*C*HHCH_2CH_2N), 3.81 (3 H, s, NCH_3), 3.68 (1 H, ddd, *J* 10.7, 6.2, 5.0, OCH*H*CH_2CH_2N), 2.11 – 1.99 (2 H, m, $OCH_2CH_2CH_2N$); **^{13}C NMR** δ_C (101 MHz, Acetonitrile-d_3) 168.08, 166.26, 166.05, 165.99 (4 C=O), 136.96 (NCHN), 134.63, 134.51, 130.32, 130.07, 129.97, 129.82, 129.75, 129.55 (C_{arom}), 124.44 (N*C*HCHN), 123.33 (NCH*C*HN), 101.22 (C-1), 74.17 (C-3), 72.82 (C-2), 72.47 (C-5), 69.98 (C-4), 66.92 ($OCH_2CH_2CH_2N$), 64.25 (C-6), 47.50 ($OCH_2CH_2CH_2N$), 41.93 (CH_2Cl), 36.76 (NCH_3), 30.38 ($OCH_2CH_2CH_2N$); **m/z** (ESI-HRMS) $C_{36}H_{36}ClN_2O_{10}^+$ ([M - OTf]$^+$) calculated 691.2053; found 691.2077; (TLC-MS- (ESI)) $CF_3O_3S^-$ ([OTf]$^-$) calculated 148.9; found 149.0; **IR** ν_{max}/cm^{-1} 3155w, 3116w, 2961w, 1731s (C=O), 1601w, 1452, 1318, 1261s, 1226, 1163, 1109, 1096, 1070, 1030, 712, 639; $[\alpha]_D^{23}$ + 4 [*c* 0.96, DCM].

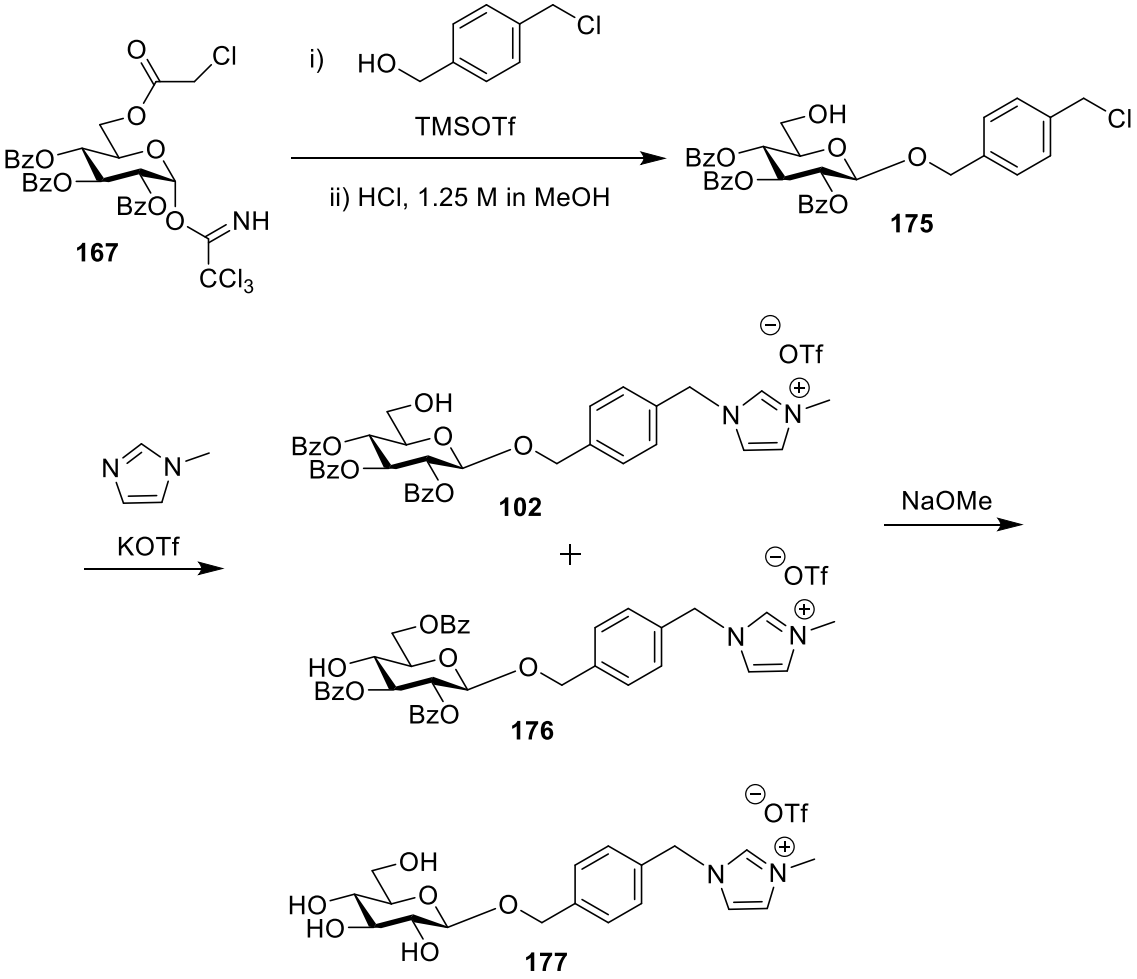
4-(1-Methyl-3-methyleneimidazolium)benzyl 2,3,4-tri-*O*-benzoyl-6-*O*-chloroacetyl-β-D-glucopyranoside trifluoromethanesulfonate 174



Following the general procedure for I-Tag glycosylations in flow using glycosyl acceptor 1-(4-(hydroxymethyl)benzyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate **157** (0.0564 g, 0.160 mmol, 1 eq) and glycosyl donor 2,3,4-tri-*O*-benzoyl-6-*O*-chloroacetyl-α-D-glucopyranosyl trichloroacetimidate **167** (0.2283 g, 0.320 mmol, 2.0 eq). 0.80 mL of anhydrous acetonitrile was added to the donor/acceptor vial, resulting in a solution of volume 0.93 mL and therefore approximately 0.172 M in acceptor and 0.344 M in donor. A stock solution of TMSOTf (0.06 M in MeCN) was made by dissolving TMSOTf (0.109 mL, 0.600 mmol) in 10 mL anhydrous MeCN. 1.0 mL of this stock solution was taken into a syringe and used for this reaction. The flow reaction was performed at RT. Reaction solution was collected for a total of 12 min 30 sec. The crude product was dissolved in DCM (5 mL) and washed with water (5 mL), then the water was extracted with further portions of DCM (2 x 5 mL). The dried residue was washed with Et_2O (2 x 6 mL), DCM:Et_2O 5:95 (3 x 6 mL) and DCM:Et_2O 1:9 (4 x 6 mL) then dried under reduced pressure to yield the title compound **174** as a single anomer (0.0950 g, 75 %); **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 9.16 (1 H, s, NCHN), 7.93 – 7.87 (4 H, m, H_{arom}), 7.81 – 7.78 (2 H, m, H_{arom}), 7.56 – 7.48 (2 H, m, H_{arom}), 7.44 – 7.33 (5 H, m, H_{arom}), 7.29 – 7.21 (7 H, m, H_{arom}, NC*H*CHN), 7.17 (1 H, t, *J* 1.8, NCHC*H*N), 5.86 (1 H, t, *J* 9.7, H-3), 5.58 (1 H, t, *J* 9.7, H-4), 5.55 (1 H, dd, *J* 9.8, 7.9, H-2), 5.28 (2 H, s, NCH_2), 4.92 – 4.88 (2 H, m, H-1, (C-1)OC*H*H), 4.69 (1 H, d, *J* 12.6, (C-1)OCH*H*), 4.43 –

4.40 (2 H, m, H-6a, H-6b), 4.10 (2 H, s, CH_2Cl), 4.09 – 4.03 (1 H, m, H-5), 3.90 (3 H, s, NCH_3); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) 167.21 (C=O (ClAc)), 165.83, 165.40, 165.28 (3 C=O (Bz)), 138.56 (4˚ $C_{arom}CH_2O$(C-1)), 137.11 (NCHN), 133.80, 133.67, 133.47 (C_{arom}), 132.32 (4˚ $C_{arom}CH_2N$), 129.94, 129.88, 129.84, 129.17, 129.15, 128.81, 128.78, 128.67, 128.65, 128.63, 128.46 (C_{arom}), 123.68 (N*C*HCHN), 122.04 (NCH*C*HN), 100.18 (C-1), 72.77 (C-3), 72.13 (C-5), 71.89 (C-2), 70.52 ((C-1)OCH_2), 69.30 (C-4), 63.93 (C-6), 53.26 (NCH_2), 40.87 (CH_2Cl), 36.58 (NCH_3); **m/z** (ESI-HRMS) $C_{41}H_{38}ClN_2O_{10}^+$ ([M - OTf]$^+$) calculated 753.2209; found 7533.2208; (TLC-MS- (ESI)) $CF_3O_3S^-$ ([OTf]$^-$) calculated 148.9; found 149.0; **IR** ν_{max}/cm^{-1} 3151w, 3072w, 2960w, 1729 (C=O), 1601, 1583w, 1492w, 1452, 1412w, 1365w, 1315, 1259s, 1225, 1159, 1106, 1095, 1070, 1030, 978, 939w, 853w, 803w, 756w, 711, 688w, 638, 623, 573, 518; $[\alpha]_D^{22}$ - 12 [*c* 2.32, DCM].

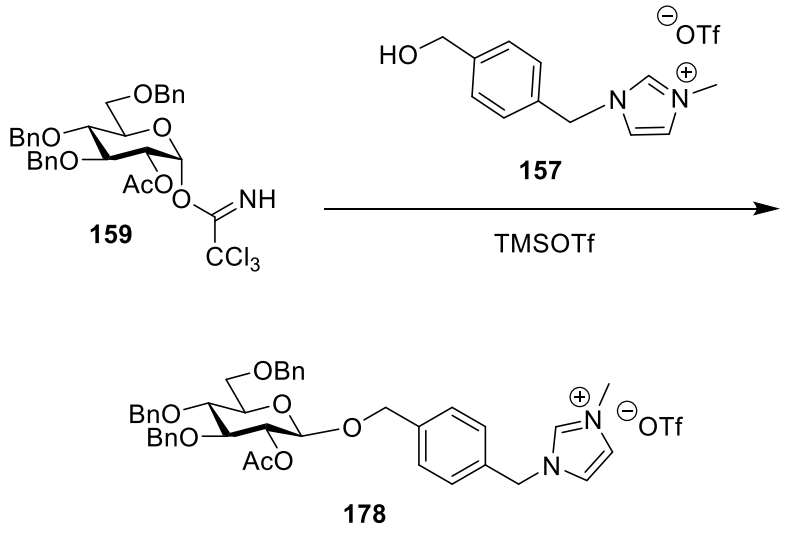
4-(1-Methyl-3-methyleneimidazolium)benzyl β-D-glucopyranoside trifluoromethanesulfonate 177



Following the general procedure for I-Tag glycosylations in flow using glycosyl acceptor 4-(chloromethyl)benzyl alcohol (0.0689 g, 0.440 mmol, 1 eq) and glycosyl donor 2,3,4-tri-*O*-benzoyl-6-*O*-chloroacetyl-α-D-glucopyranosyl trichloroacetimidate **167** (0.6277 g, 0.880 mmol, 2.0 eq). 2.20 mL of anhydrous DCM was added to the donor/acceptor vial, resulting in a solution of volume 2.75 mL and therefore approximately 0.160 M in acceptor and 0.320 M in donor. A stock solution of TMSOTf (0.06 M in DCM) was made by dissolving TMSOTf (0.217 mL, 1.200 mmol) in 20 mL anhydrous DCM. 3.0 mL of this stock solution was taken into a syringe and used for this reaction. The flow reaction was performed at RT. Reaction solution was collected for a total of 41 min 30 sec. The crude product was collected in DCM (20 mL) and washed with water (8 mL), then the water was extracted with a further portion of DCM (20 mL). The dried residue was dissolved in a minimal volume of DCM, then HCl, 1.25 M in MeOH (7.04 mL, 8.800 mmol) was added. The resulting solution was stirred for 16 h at RT in air, then diluted with DCM (15 mL) and water (10 mL) and product was extracted into the DCM phase. The aqueous phase was washed with a further DCM portion (15 mL) then DCM washings were combined, dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. The dried residue was washed with hexane three times to give impure intermediate **175**. Intermediate **175** was dried under reduced pressure for 1 h, before being dissolved in anhydrous MeCN (5 mL) under a nitrogen atmosphere. 1-Methyl imidazole (0.14 mL, 1.76 mmol) and potassium trifluoromethanesulfonate (0.3312 g, 1.76 mmol) were added and the resulting mixture was heated under reflux at 90 ˚C and stirred for 18 h, after which time TLC (DCM:MeOH 94:6) showed the reaction to be complete and suggested neighbouring group migration having occurred. Solvent was removed under reduced pressure, then DCM and 1 M $HCl_{(aq.)}$ were added to the residue and product was extracted into the DCM phase. The aqueous phase was washed with DCM twice more, then DCM portions were combined, dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. The crude residue was washed with Et_2O once and DCM:Et_2O 5:95 twice to give a mixture of I-Tagged products **102** and **176**. This mixture was dissolved in methanol (2.2 mL) and sodium methoxide (50.0 μL, 0.22 mmol, 25 % wt in MeOH) was added. The solution was stirred at RT for 3 h after which time TLC-MS showed the reaction to be complete. The solution was then brought to pH 7, as monitored by universal indicator paper, using 1 M $HCl_{(aq.)}$. Solvent was removed under reduced pressure, then the residue was diluted with DCM and water and product was extracted into the aqueous phase.

Water was removed under reduced pressure. The crude product was purified by reverse-phase HPLC (Water:MeCN) to yield the title compound **177** (0.1110 g, 49 % over 4 steps) as a solid; **^{1}H NMR** δ_H (500 MHz, Methanol-d_4) 8.95 (1 H, s, NCHN), 7.58 (1 H, d, *J* 2.0, NC*H*CHN), 7.56 (1 H, d, *J* 2.0, NCHC*H*N), 7.53 – 7.48 (2 H, m, H_{arom}), 7.43 – 7.38 (2 H, m, H_{arom}), 5.39 (2 H, s, NCH_2), 4.94 (1 H, d, *J* 12.3, (C-1)OC*H*H), 4.70 (1 H, d, *J* 12.3, (C-1)OCH*H*), 4.35 (1 H, dd, *J* 7.7, 0.9, H-1), 3.92 (3 H, s, NCH_3), 3.89 (1 H, dd, *J* 12.0, 2.1, H-6a), 3.68 (1 H, dd, *J* 11.8, 5.5, H-6b), 3.37 – 3.23 (4 H, m, H-2, H-3, H-4, H-5); **^{13}C NMR** δ_C (126 MHz, Methanol-d_4) 140.64 (4˚ $C_{arom}CH_2O$(C-1)) , 134.44 (4˚ $C_{arom}CH_2N$), 129.91, 129.65 (C_{arom}), 125.21 (NCH*C*HN), 123.60 (N*C*HCHN), 103.41 (C-1), 78.10, 78.04, 75.11, 71.66 (C-2, C-3, C-4, C-5), 71.07 ((C-1)O*C*H_2), 62.77 (C-6), 53.83 (NCH_2), 36.52 (NCH_3); **m/z** (ESI-HRMS) $C_{18}H_{25}N_2O_6^+$ ([M – OTf]$^+$) calculated: 365.1707; found 365.1712; (TLC-MS- (ESI)) $CF_3O_3S^-$ ([OTf]$^-$) calculated 149.0; found 148.8; **IR** ν_{max}/cm^{-1} 3419br (OH), 3156w, 3113w, 2968w, 2929w, 1577, 1452, 1416, 1253s, 1225, 1160, 1076, 1028s, 758, 638, 574, 517; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ - 24 [*c* 1.08, MeOH].

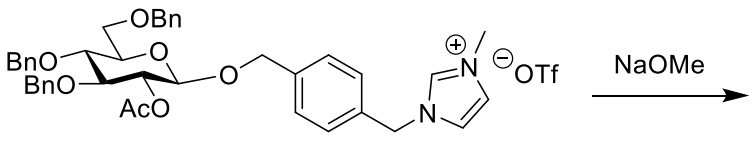
4-(1-Methyl-3-methyleneimidazolium)benzyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranoside trifluoromethanesulfonate 178



Following the general procedure for I-Tag glycosylations in flow using glycosyl acceptor 1-(4-(hydroxymethyl)benzyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate **157** (0.1409 g, 0.400 mmol, 1 eq) and glycosyl donor 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl trichloroacetimidate **159** (0.5060 g, 0.800 mmol, 2.0 eq). 2.00 mL of anhydrous MeCN was added to the donor/acceptor vial, resulting in a solution of volume 2.48 mL and therefore approximately 0.161 M in acceptor and 0.322 M in

donor. A stock solution of TMSOTf (0.06 M in MeCN) was made by dissolving TMSOTf (0.217 mL, 1.200 mmol) in 20 mL anhydrous MeCN. 2.6 mL of this stock solution was taken into a syringe and used for this reaction. Reaction solution was collected for a total of 33 min 15 sec. The crude product was dissolved in DCM (20 mL) and washed with water (8 mL), then the water was extracted with DCM (20 mL) and DCM portions were combined. The dried residue was washed with Et_2O (7 x 8 mL), then dried under reduced pressure to yield the title compound **178** as a single anomer (0.2604 g, 84 %); **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 9.03 (1 H, s, NCHN), 7.36 – 7.22 (19 H, m, H_{arom}), 7.21 – 7.17 (2 H, m, H_{arom}), 5.26 (2 H, s, NCH_2), 5.01 (1 H, t, *J* 8.5, H-2), 4.85 (1 H, d, *J* 12.3, (C-1)OC*H*H), 4.80 (2 H, app dd, *J* 11.1, 3.0, PhCH_2), 4.67 (1 H, d, *J* 11.4, PhCH*H*), 4.61 – 4.54 (3 H, m, PhCH_2), 4.52 (1 H, d, *J* 12.1, PhCH*H*), 4.47 (1 H, d, *J* 7.9, H-1), 3.81 (3 H, s, NCH_3), 3.80 – 3.73 (2 H, m, H-6a, H-6b), 3.72 (1 H, d, *J* 9.2, H-4), 3.68 (1 H, t, *J* 9.0, H-3), 3.51 (1 H, ddd, *J* 9.4, 4.2, 2.2, H-5), 1.96 (3 H, s, (C=O)CH_3); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) 169.82 (C=O), 138.89 (4˚ *C*$_{arom}CH_2O$(C-1)), 138.12, 138.01, 137.88 (3 4˚ C_{arom} (Bn)), 136.58 (NCHN), 132.44 (4˚ *C*$_{arom}CH_2N$), 128.96, 128.51, 128.47, 128.30, 127.99, 127.95, 127.89, 127.87, 127.83, 127.80 (C_{arom}), 123.82 (N*C*HCHN), 122.18 (NCH*C*HN), 100.34 (C-1), 82.85 (C-3), 77.93 (C-4), 75.23, 75.12, 75.06 (C-5, 2 Ph*C*H_2), 73.52 (Ph*C*H_2), 73.23 (C-2), 70.11 ((C-1)O*C*H_2), 68.64 (C-6), 52.98 (NCH_2), 36.32 (NCH_3), 20.96 ((C=O)*C*H_3); **m/z** (ESI-HRMS) $C_{41}H_{45}N_2O_7^+$ ([M – OTf]$^+$) calculated: 677.3221; found 677.3211; (TLC-MS- (ESI)) $CF_3O_3S^-$ ([OTf]$^-$) calculated 149.0; found 148.8; **IR** ν_{max}/cm^{-1} 3153w, 3031w, 2918w, 2873, 1744, 1454, 1365, 1259s, 1227, 1154, 1061, 1031s, 736, 700, 638; $[\boldsymbol{\alpha}]_D^{25}$ - 10 [*c* 1.28, DCM].

4-(1-Methyl-3-methyleneimidazolium)benzyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranoside trifluoromethanesulfonate 179



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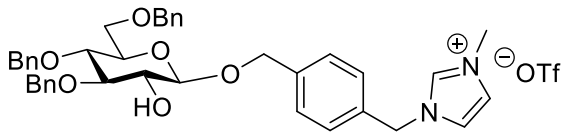
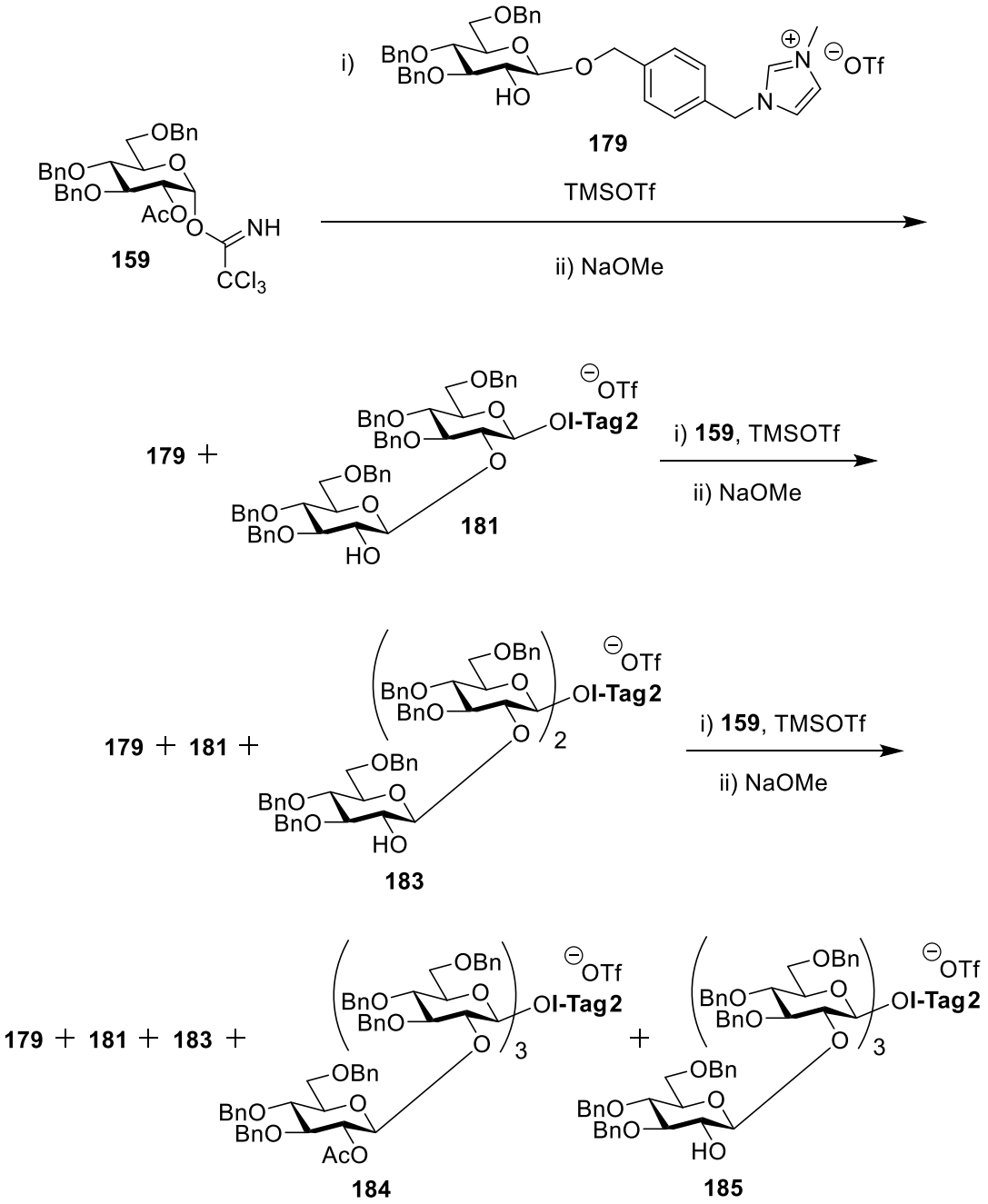


179

4-(1-Methyl-3-methyleneimidazolium)benzyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranoside trifluoromethanesulfonate **178** (0.2562 g, 0.310 mmol) was dissolved in methanol (1.55 mL) and sodium methoxide (14.2 µL, 0.062 mmol, 25 % wt in MeOH) was added. The solution was stirred at RT for 19 h after which time TLC-MS showed the reaction to be complete. The solution was then brought to pH 7, as monitored by universal indicator paper, using 1 M $HCl_{(aq.)}$. Most methanol solvent was removed under reduced pressure, then the residue was diluted with DCM and washed with water. The aqueous phase was washed with two further portions of DCM and the DCM portions were combined, dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. The dried residue was washed with Et_2O (2 x 8 mL), then acetonitrile was added and the mixture was filtered. Solvent was removed under reduced pressure to yield the title compound **179** (0.1817 g, 75 %); **^{1}H NMR** δ_H (500 MHz, Acetonitrile-d_3) 8.75 (1 H, s, NCHN), 7.46 (2 H, app d, *J* 7.9, H_{arom}), 7.40 – 7.25 (20 H, m, H_{arom}), 7.23 – 7.20 (2 H, m, H_{arom}), 5.31 (2 H, s, NCH_2), 4.92 (1 H, d, *J* 11.3, PhC*H*H), 4.85 (1 H, d, *J* 12.4, (C-1)OC*H*H), 4.78 (2 H, app dd, *J* 11.1, 8.5, PhCH_2), 4.65 (1 H, d, *J* 12.4, (C-1)OCH*H*), 4.58 – 4.50 (3 H, m, PhCH_2), 4.40 (1 H, d, *J* 7.7, H-1), 3.79 (3 H, s, NCH_3), 3.74 (1 H, dd, *J* 10.9, 1.8, H-6a), 3.69 (1 H, dd, *J* 10.9, 4.1, H-6b), 3.57 – 3.46 (3 H, m, H-3, H-4, H-5), 3.43 (1 H, t, *J* 8.2, H-2); **^{13}C NMR** δ_C (126 MHz, Acetonitrile-d_3) 140.12, 140.08, 139.53, 139.47 (4 4˚ C_{arom}) , 137.22 (NCHN), 134.13 (4˚ C_{arom}), 129.50, 129.43, 129.25, 129.17, 129.12, 128.82, 128.72, 128.71, 128.49, 128.47, 128.30 (C_{arom}), 124.86 (N*C*HCHN), 123.10 (NCH*C*HN), 103.11 (C-1), 85.73 (C-3), 78.54 (C-4), 75.51, 75.48, 75.33 (C-2, C-5, 2 Ph*C*H_2), 73.75 (Ph*C*H_2), 70.81 ((C-1)O*C*H_2), 69.93 (C-6), 53.30 (NCH_2), 36.83 (NCH_3); **m/z** (ESI-HRMS) $C_{39}H_{43}N_2O_6^+$ ([M – OTf]$^+$) calculated: 635.3116; found 635.3098; (TLC-MS- (ESI)) $CF_3O_3S^-$ ([OTf]$^-$) calculated 149.0; found 148.8; **IR** ν_{max}/cm^{-1} 3440br, 3031w, 2869, 1573, 1497, 1454, 1360, 1256, 1224, 1156, 1112, 1055s, 1028s, 910, 826, 732s, 698, 637s, 623, 573, 517, 463; $[\alpha]_D^{23}$ - 3 [*c* 1.55, DCM].

4-(1-Methyl-3-methyleneimidazolium)benzyl 3,4,6-tri-*O*-benzyl-2-*O*-(3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-β-D-glucopyranoside trifluoromethanesulfonate 181, 4-(1-methyl-3-methyleneimidazolium)benzyl 3,4,6-tri-*O*-benzyl-2-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-(3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranoside trifluoromethanesulfonate 183, 4-(1-methyl-3-methyleneimidazolium)benzyl 3,4,6-tri-*O*-benzyl-2-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-

D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranoside trifluoromethanesulfonate 184 and 4-(1-methyl-3-methyleneimidazolium)benzyl 3,4,6-tri-*O*-benzyl-2-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-(3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-glucopyranoside trifluoromethanesulfonate 185

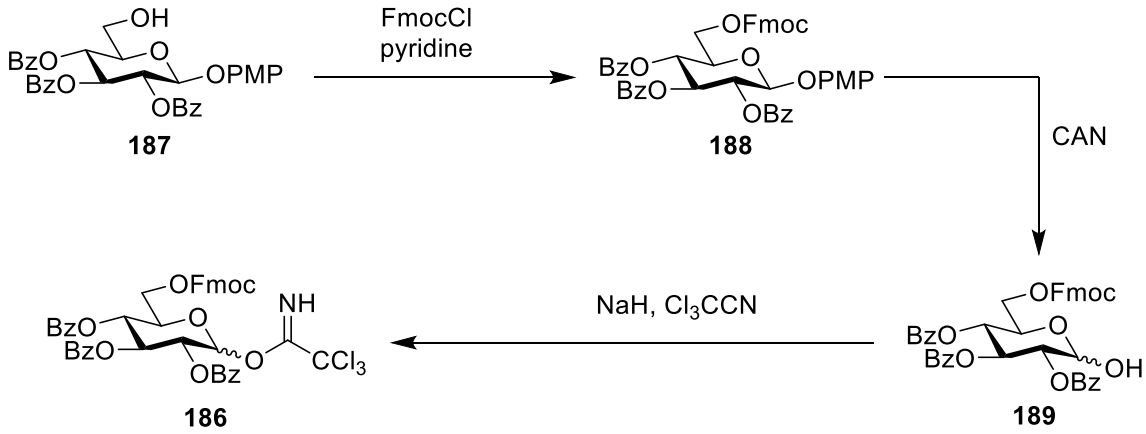


Following the general procedure for I-Tag glycosylations in flow using glycosyl acceptor 4-(1-methyl-3-methyleneimidazolium)benzyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranoside trifluoromethanesulfonate **179** (0.2113 g, 0.269 mmol, 1 eq) and glycosyl donor 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl trichloroacetimidate **159** (0.3427 g, 0.538 mmol, 2.0 eq). 1.35 mL of anhydrous MeCN was added to the donor/acceptor vial, resulting in a solution of volume 1.70 mL and therefore approximately 0.158 M in acceptor and 0.316 M in donor. A stock solution of TMSOTf (0.06 M in MeCN) was made by dissolving TMSOTf (0.217 mL, 1.200 mmol) in 20 mL anhydrous MeCN. 2.0 mL of this stock solution was taken into a syringe and used for this reaction. The flow reaction was performed at RT. Reaction solution was collected for a total of 25 min into reagent grade DCM, then solvent was removed under reduced pressure. The crude residue was washed with hexane:Et_2O 1:1 (4 x 4 mL), then the product mixture was analysed by ^1H NMR spectroscopy, revealing a mixture of **179**:**180** in a 45:55 ratio. The product mixture was dissolved in a minimal volume of methanol and sodium methoxide (46.2 µL, 0.202 mmol, 25 % wt in MeOH) was added. The solution was stirred at RT for 24 h after which time TLC-MS showed the reaction to be complete. The solution was then brought to pH 7, as monitored by universal indicator paper, using 1 M $HCl_{(aq.)}$. The reaction solution was diluted with DCM and washed with water. The aqueous phase was washed with two further portions of DCM and the DCM portions were combined, dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. The dried residue was washed with hexane:Et_2O 1:3 (8 mL) and hexane:Et_2O 1:1 (4 x 8 mL), then dried under reduced pressure to give 0.2003 g of a 45:55 mixture of **179**:**181** which were used as the glycosyl acceptors in the next flow reaction. Following the general procedure for I-Tag glycosylations in flow using glycosyl acceptors **179** and **181** (0.2003 g) and glycosyl donor 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl trichloroacetimidate **159** (0.2510 g, 0.394 mmol). 0.96 mL of anhydrous MeCN was added to the donor/acceptor vial, resulting in a solution of volume 1.25 mL. 1.50 mL of the TMSOTf (0.06 M in MeCN) stock solution was taken into a syringe and used for this reaction. The flow reaction was performed at RT. Reaction solution was collected for a total of 17 min 15 sec into reagent grade DCM, then solvent was removed under reduced pressure. The crude residue was analysed by TLC-MS, revealing a mixture of monosaccharide **179**, disaccharides **180** and **181** and trisaccharide **182**. The product mixture was dissolved in a minimal volume of methanol and sodium methoxide (33.8 µL, 0.148 mmol, 25 % wt in MeOH) was added. The

solution was stirred at RT for 18 h, but TLC-MS showed the reaction was not yet complete. A further portion of sodium methoxide (67.6 µL, 0.296 mmol, 25 % wt in MeOH) was added and the reaction mixture was stirred at RT for a further 24 h, then another portion of sodium methoxide (22.3 µL, 0.098 mmol, 25 % wt in MeOH) was added and the reaction mixture was stirred at RT for a further 6 h, to allow complete deacetylation. The solution was then brought to pH 7, as monitored by universal indicator paper, using 1 M HCl $_{(aq.)}$. The reaction solution was diluted with DCM and washed with water. The aqueous phase was washed with two further portions of DCM and the DCM portions were combined, dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. The dried residue was washed with hexane:Et_2O 1:1 (3 x 8 mL), then dried under reduced pressure to give 0.2341 g of a mixture of **179**:**181**:**183**, which were used as the glycosyl acceptors in the next flow reaction. Following the general procedure for I-Tag glycosylations in flow using glycosyl acceptors **179**, **181** and **183** (0.2341 g) and glycosyl donor 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl trichloroacetimidate **159** (0.2038 g, 0.320 mmol). 0.80 mL of anhydrous MeCN was added to the donor/acceptor vial, resulting in a solution of volume 1.18 mL. 1.50 mL of the TMSOTf (0.06 M in MeCN) stock solution was taken into a syringe and used for this reaction. The flow reaction was performed at RT. Reaction solution was collected for a total of 16 min 20 sec into reagent grade DCM, then solvent was removed under reduced pressure. The crude residue was analysed by TLC-MS, revealing a mixture of monosaccharide **179**, disaccharides **180** and **181**, trisaccharides **182** and **183** and tetrasaccharide **184**. The crude residue was washed with hexane:Et_2O 2:1 (2 x 10 mL), then dissolved in a minimal volume of methanol before sodium methoxide (91.4 µL, 0.400 mmol, 25 % wt in MeOH) was added. The solution was stirred at RT for 19 h, at which point TLC-MS showed that deacetylation was complete for the di- and trisaccharides, but not for the tetrasaccharide. A further portion of sodium methoxide (500.0 µL, 2.188 mmol, 25 % wt in MeOH) was added and the reaction mixture was stirred at RT for a further 53 h, however, complete deprotection of tetrasaccharide **184** was not possible. The solution was then brought to pH 7, as monitored by universal indicator paper, using 1 M HCl $_{(aq.)}$. The reaction solution was diluted with DCM and washed with water. The aqueous phase was washed with two further portions of DCM and the DCM portions were combined, dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. The dried residue was washed with hexane:Et_2O 1:1 (6 x 8 mL) and hexane:Et_2O 1:2 (2 x 8 mL), then dried under reduced pressure

to give 0.1370 g of a mixture of **179**:**181**:**183**:**184**:**185**. This mixture was only analysed by MS and was not separated, therefore further experimental details could not be acquired.

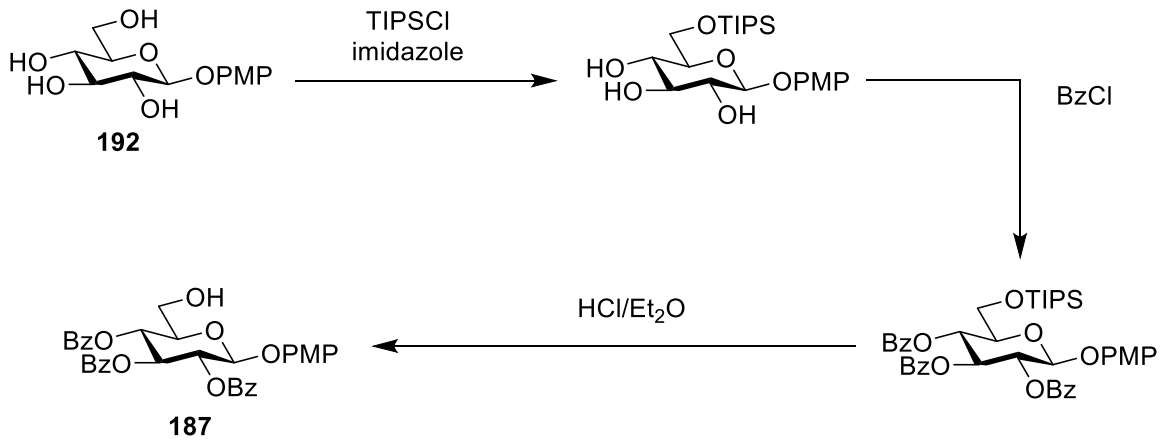
2,3,4-Tri-*O*-benzoyl-6-*O*-(9-fluorenylmethoxycarbonyl)-D-glucopyranosyl trichloroacetimidate 186



4-Methoxyphenyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside **187** (0.250 g, 0.418 mmol) was dried for 1 h under vacuum before being dissolved in anhydrous DCM (3.7 mL) under a nitrogen atmosphere. The resulting solution was cooled to 0 °C then anhydrous pyridine (92.9 µL, 1.15 mmol) was added. Fluorenylmethyloxycarbonyl chloride (0.216 g, 0.835 mmol) was added in small portions over 30 min with stirring. Following complete fluorenylmethyloxycarbonyl chloride addition, the resulting solution was stirred for 2 h at RT, after which time TLC (Hexane:EtOAc 7:3) showed the reaction to be complete. Methanol (0.05 mL) was added to quench excess fluorenylmethyloxycarbonyl chloride, then solvent was removed under reduced pressure, with co-evaporation with toluene (3 x 1 mL). The residue was dissolved in DCM (5 mL) and washed with water (2 x 3 mL), $NaHCO_{3\ (sat.\ aq.)}$ (3 mL) and brine (3 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. Following column chromatography (Hexane:EtOAc 8:2), 4-methoxyphenyl 2,3,4-tri-*O*-benzoyl-6-*O*-(9-fluorenylmethoxycarbonyl)-β-D-glucopyranoside **188** (0.284 g, 0.345 mmol, 83 %) was obtained. This product was dissolved in a mixture of DCM:MeCN (10 mL, 1:9) at RT. To this solution water (1 mL) was added such that, with vigorous stirring, the resulting solution consists of a single phase. Ceric ammonium nitrate (0.379 g, 0.691 mmol) was added and the solution stirred for 5.5 h at RT, after which time TLC (Hexane:EtOAc 6:4) and TLC-MS ($C_{42}H_{34}O_{11}Na^+$ ([M + Na]$^+$) calculated 737.2; found 737.5)

showed the reaction to be complete. Most of the solvent was removed under reduced pressure, and the residue was diluted with EtOAc (30 mL). The resulting solution was washed with water (2 x 20 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. Following column chromatography (Hexane:EtOAc 7:3) the free hemiacetal intermediate **189** (0.194 g, 0.271 mmol, 78 %) was obtained. This intermediate was dried for 1 h under vacuum before being dissolved in a mixture of anhydrous DCM:trichloroacetonitrile (10 mL, 1:1) under a nitrogen atmosphere. To this solution, sodium hydride (60 % in mineral oil, 0.0033 g, 0.0813 mmol) was added and the resulting solution was stirred overnight at RT under a nitrogen atmosphere, after which time TLC (Hexane:EtOAc 8:2) showed the reaction to be complete, with the product present as two anomers. Silica was added, then the solvent was removed under reduced pressure to dry load the product. Following column chromatography (Hexane:EtOAc 8:2), the title compound **186** (0.230 g, 99 %, α:β = 1:0.85) was obtained as two anomers that could be partially separated by column chromatography. Only the α anomer could be obtained in sufficiently high purity for complete characterisation. Note that only characteristic, non-overlapping, distinguishable NMR spectroscopy signals are given for the β anomer; **^{1}H NMR α anomer**: δ_H (500 MHz, Chloroform-*d*) 8.65 (1 H, s, NH), 7.97 (4 H, td, *J* 8.0, 1.4, H_{arom}), 7.88 (2 H, dd, *J* 8.3, 1.4, H_{arom}), 7.77 (2 H, d, *J* 7.5, H_{arom}), 7.62 (2 H, dd, *J* 15.2, 7.5, H_{arom}), 7.54 – 7.49 (2 H, m, H_{arom}), 7.47 – 7.28 (11 H, m, H_{arom}), 6.86 (1 H, d, *J* 3.7, H-1), 6.28 (1 H, t, *J* 9.9, H-3), 5.77 (1 H, t, *J* 10.0, H-4), 5.64 (1 H, dd, *J* 10.2, 3.7, H-2), 4.58 (1 H, dt, *J* 10.3, 3.8, H-5), 4.45 – 4.43 (2 H, m, H-6a, H-6b), 4.41 (1 H, dd, *J* 10.3, 7.5, COC*H*HCH), 4.32 (1 H, dd, *J* 10.3, 7.7, COCH*H*CH), 4.26 (1 H, t, *J* 7.5, $COCH_2CH$); **β anomer**: δ_H (400 MHz, Chloroform-*d*) 6.26 (1 H, d, *J* 7.6, H-1); **^{13}C NMR α anomer**: δ_C (126 MHz, Chloroform-*d*) 165.79, 165.52, 165.39 (3 C=O (Bz)), 160.63 (CNH), 154.92 (C=O (Fmoc)), 143.57, 143.37, 141.40, 141.37 (4° C_{arom}), 133.76, 133.71, 133.47, 130.07, 130.05, 129.86, 128.98, 128.73, 128.67, 128.63, 128.58, 128.50, 128.02, 127.36, 125.51, 125.40, 120.15, 120.14 (C_{arom}), 93.23 (C-1), 90.82 (CCl_3), 70.76, 70.63, 70.49 (C-2, C-5, $COCH_2CH$), 70.21 (C-3), 68.64 (C-4), 65.62 (C-6), 46.77 ($COCH_2CH$); **β anomer**: δ_C (126 MHz, Chloroform-*d*) 95.91 (C-1); **m/z α anomer**: $C_{44}H_{34}Cl_3NO_{11}Na^+$ ($[M + Na]^+$) calculated 880.1090; found 880.1070; $C_{42}H_{33}O_{10}^+$ ($[M – OC(NH)CCl_3]^+$) calculated 697.2068; found 697.2060; **IR α anomer**: ν_{max}/cm^{-1} 3468w, 3350w, 3069w, 2957w, 2923w, 1727s (C=O), 1678, 1601, 1451, 1316, 1259s, 1178, 1160, 1105, 1093, 1069, 1026, 970, 825, 795, 760, 742, 708s, 686; **α anomer**: $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{26}}$ + 10 [*c* 1.06, DCM].

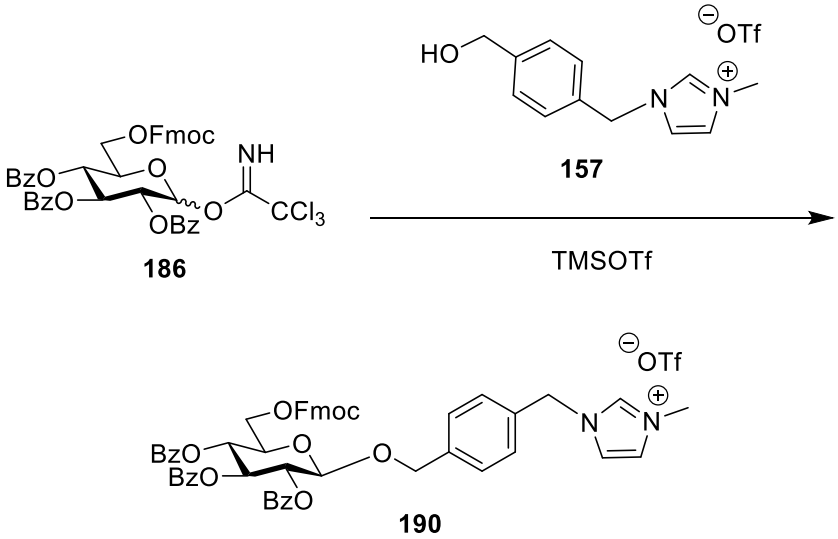
4-Methoxyphenyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside 187



4-Methoxyphenyl β-D-glucopyranoside **192** (7.33 g, 25.62 mmol) was dried for 1 h under vacuum before being dissolved in anhydrous pyridine (125 mL) under a nitrogen atmosphere. To this solution, imidazole (3.49 g, 51.24 mmol) was added and the solution was cooled to 0 °C. Triisopropylsilyl chloride (6.59 mL, 30.74 mmol) was added dropwise and the reaction was stirred at RT for 18 h. After this time, TLC (DCM:MeOH 95:5) showed the silylation reaction to be complete. The solvent was removed under reduced pressure and the residue was dissolved in DCM (150 mL). The DCM phase was washed with water (2 x 100 mL) and the resulting aqueous phase was furthered washed with DCM (100 mL), then DCM washings were combined. The combined organic phase was dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (DCM:MeOH 99:1 → 96:4 → 95:5) to give 4-methoxyphenyl 6-*O*-triisopropylsilyl-β-D-glucopyranoside (10.04 g, 89 %), with identity and purity confirmed by ^{1}H NMR spectroscopy. 4-Methoxyphenyl 6-*O*-triisopropylsilyl-β-D-glucopyranoside (5.85 g, 13.22 mmol) was dried for 1 h under vacuum before being dissolved in anhydrous pyridine (80 mL) under a nitrogen atmosphere. Benzoyl chloride (12.29 mL, 105.79 mmol) was added, and the solution was stirred at RT for 17 h under a nitrogen atmosphere. After this time, TLC (Hexane:EtOAc 8:2) showed the benzoylation reaction to be complete. Methanol (15 mL) was cautiously added to quench excess benzoyl chloride, then solvent was removed under reduced pressure. The residue was dissolved in DCM (100 mL) and the resulting organic solution was washed with 1 M HCl $_{(aq.)}$ (2 x 75 mL), $NaHCO_3$ $_{(sat.\ aq.)}$ (75 mL) and water (75 mL), dried using magnesium sulfate, filtered and the solvent was

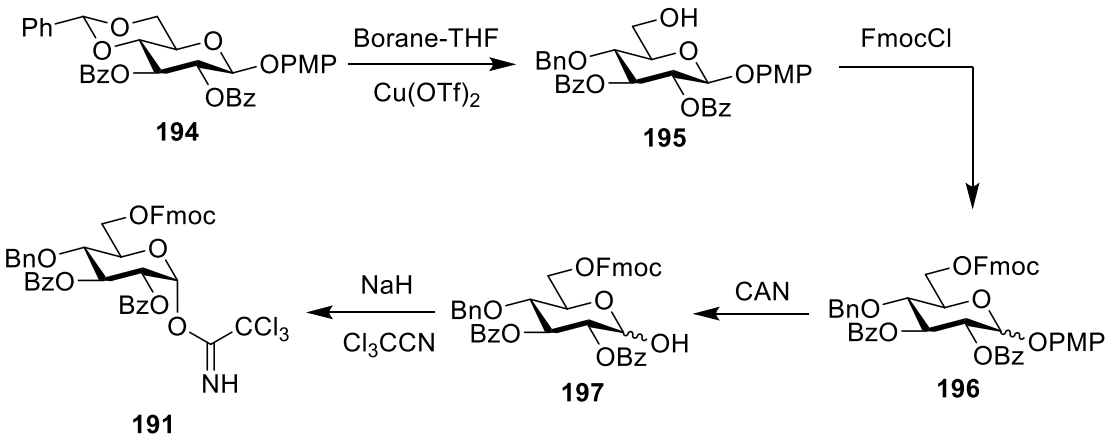
removed under reduced pressure. The resulting benzoylated intermediate was dissolved in MeCN (100 mL) in air at RT. HCl in diethyl ether (100 mL, 200 mmol, 2.0 M) was added and the resulting solution was stirred at RT for 2 h, after which time TLC (Hexane:EtOAc 6:4) showed the reaction to be complete. The solution was then cautiously brought to pH 7, as monitored by universal indicator paper, using 1 M NaOH $_{(aq.)}$. The ethereal phase was separated from the aqueous phase, dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (Hexane:EtOAc 8:2 → 7:3 → 6:4) to give the title compound **187** (6.75 g, 85 % over 2 steps) with spectroscopic details in accordance with the literature;[179] **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 7.99 – 7.94 (4 H, m, H_{arom}), 7.89 – 7.84 (2 H, m, H_{arom}), 7.57 – 7.35 (7 H, m, H_{arom}), 7.32 – 7.27 (2 H, m, H_{arom}), 6.99 – 6.93 (2 H, m, H_{arom}), 6.82 – 6.76 (2 H, m, H_{arom}), 6.00 (1 H, t, *J* 9.7, H-3), 5.75 (1 H, dd, *J* 9.8, 7.9, H-2), 5.58 (1 H, t, *J* 9.7, H-4), 5.30 (1 H, d, *J* 7.9, H-1), 3.93 – 3.86 (2 H, m, H-5, H-6a), 3.82 – 3.76 (1 H, m, H-6b), 3.75 (3 H, s, CH_3); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) 166.11, 165.96, 165.22 (3 C=O), 155.93, 151.12 (2 4° C_{arom}), 133.86, 133.45, 130.09, 129.94, 129.92, 129.31, 128.93, 128.67, 128.63, 128.60, 128.55, 128.48 (C_{arom} (Bz)), 118.98, 114.75 (C_{arom} (PMP)), 100.96 (C-1), 75.06 (C-5), 72.86 (C-3), 71.93 (C-2), 69.54 (C-4), 61.56 (C-6), 55.76 (CH_3); **m/z** (TLC-MS+ (ESI)) $C_{34}H_{30}O_{10}Na^+$ ($[M + Na]^+$) calculated 621.2; found 621.2; $C_{36}H_{33}NO_{10}Na^+$ ($[M + MeCN + Na]^+$) calculated 662.2; found 662.2; $C_{68}H_{60}O_{20}Na^+$ ($[2M + Na]^+$) calculated 1219.4; found 1219.1.

4-(1-Methyl-3-methyleneimidazolium)benzyl 2,3,4-tri-*O*-benzoyl-6-*O*-(9-fluorenylmethoxycarbonyl)-β-D-glucopyranoside trifluoromethanesulfonate 190



Following the general procedure for I-Tag glycosylations in flow using glycosyl acceptor 1-(4-(hydroxymethyl)benzyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate **157** (0.0289 g, 0.082 mmol, 1 eq) and glycosyl donor 2,3,4-tri-*O*-benzoyl-6-*O*-(9-fluorenylmethoxycarbonyl)-D-glucopyranosyl trichloroacetimidate **186** (0.1404 g, 0.163 mmol, 2.0 eq). 0.41 mL of anhydrous acetonitrile was added to the donor/acceptor vial, resulting in a solution of volume 0.49 mL and therefore approximately 0.167 M in acceptor and 0.334 M in donor. A stock solution of TMSOTf (0.06 M in MeCN) was made by dissolving TMSOTf (0.109 mL, 0.600 mmol) in 10 mL anhydrous MeCN. 0.75 mL of this stock solution was taken into a syringe and used for this reaction. The flow reaction was performed at RT. Reaction solution was collected for a total of 6 min in reagent grade DCM. The crude product was dissolved in DCM (5 mL) and washed with water (5 mL), then the water was extracted with further portions of DCM (2 x 5 mL). The dried residue was washed with hexane (5 mL), hexane:Et_2O 1:1 (2 x 5 mL), hexane:Et_2O 1:3 (2 x 5 mL), and Et_2O (3 x 5 mL), then dried under reduced pressure to yield the title compound **190** as a single anomer (0.0553 g, 80 %); **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 9.19 (1 H, s, NH), 7.95 – 7.90 (4 H, m, H_{arom}), 7.82 (2 H, app dd, *J* 8.4, 1.4, H_{arom}), 7.75 (2 H, app d, *J* 7.5, H_{arom}), 7.64 – 7.46 (4 H, m, H_{arom}), 7.47 – 7.16 (15 H, m, H_{arom}), 7.16 (1 H, s, NC*H*CHN), 7.07 (1 H, s, NCHC*H*N), 5.88 (1 H, t, *J* 9.6, H-3), 5.62 – 5.53 (2 H, m, H-2, H-4), 5.25 (2 H, s, NCH_2), 4.94 – 4.86 (2 H, m, H-1, (C-1)OC*H*H), 4.67 (1 H, d, *J* 12.5, (C-1)OCH*H*), 4.47 – 4.33 (4 H, m, H-6a, H-6b, O*C*H_2CH(Fmoc)), 4.22 (1 H, t, *J* 7.3, OCH_2*C*H(Fmoc)), 4.08 (1 H, dt, *J* 9.5, 4.0, H-5), 3.89 (3 H, s, NCH_3); **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 165.89, 165.41, 165.28 (3 C=O (Bz)), 154.94 (C=O (Fmoc)), 143.45, 143.34, 141.41, 141.39 4 (4˚ C_{arom}(Fmoc)), 138.64 (4˚ *C*$_{arom}CH_2$O(C-1)), 137.33 (NCHN), 132.12 (4˚ *C*$_{arom}CH_2$N), 129.99, 129.90, 129.88, 129.23, 129.16, 128.86, 128.81, 128.78, 128.64, 128.63, 128.47, 128.09, 127.37, 125.34, 125.30 (C_{arom}), 123.55 (N*C*HCHN), 121.91 (NCH*C*HN), 120.22 (C_{arom}), 100.15 (C-1), 72.87 (C-3), 72.40 (C-5), 71.91 (C-2 or C-4), 70.41 ((C-1)O*C*H_2), 70.28 (O*C*H_2CH(Fmoc)), 69.55 (C-2 or C-4), 66.29 (C-6), 53.39 (NCH_2), 46.79 (OCH_2*C*H(Fmoc)), 36.69 (NCH_3); **m/z** (ESI-HRMS) $C_{54}H_{47}N_2O_{11}^+$ ([M - OTf]$^+$) calculated 899.3174; found 899.3183; (TLC-MS- (ESI)) $CF_3O_3S^-$ ([OTf]$^-$) calculated 148.9; found 148.9; **IR** ν_{max}/cm^{-1} 3068w, 2960w, 1732 (C=O), 1602, 1581w, 1450, 1316, 1258s, 1158, 1106, 1094, 1069, 1030, 975, 853w, 761, 743, 710, 638, 621; $[\alpha]_D^{22}$ - 5 [*c* 0.63, DCM].

2,3-Di-*O*-benzoyl-4-*O*-benzyl-6-*O*-(9-fluorenylmethoxycarbonyl)-α-D-glucopyranosyl trichloroacetimidate 191

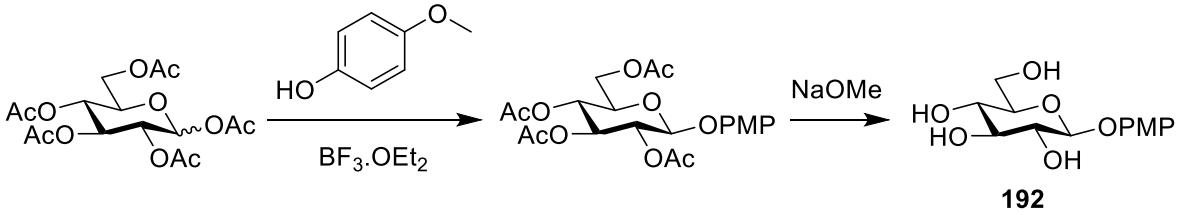


4-Methoxyphenyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside **194** (1.74 g, 2.99 mmol) was dried under vacuum for 1 h before borane-THF (20.00 mL, 20.00 mmol, 1.0 M solution in THF) was added under a nitrogen atmosphere. The mixture was stirred at RT for 10 min, then copper^{II} triflate (0.162 g, 0.448 mmol) was added, turning the cloudy off-white mixture to black over approximately 5 min. The mixture was sonicated for 1 h under a nitrogen atmosphere. The mixture stayed cloudy, indicating incomplete dissolution of starting material. To aid dissolution, approximately 5 mL of anhydrous DCM was added to the reaction mixture, then the mixture was sonicated for a further 4.5 h. After this time TLC (Hexane:EtOAc 7:3) and TLC-MS ($C_{34}H_{32}O_9Na^+$ ([M + Na]$^+$) calculated 607.2; found 607.1) showed the reaction to be complete. The reaction mixture was cooled to 0 °C, then NEt_3 (0.42 mL, 2.99 mmol) and methanol (5.4 mL) were added to quench the reaction. Solvent was then removed under reduced pressure, with co-evaporation with methanol. The crude material was purified by normal phase HPLC (Hexane:EtOAc) to give 4-methoxyphenyl 2,3-di-*O*-benzoyl-4-*O*-benzyl-β-D-glucopyranoside **195** (1.37 g), which was used directly in the next step. 4-Methoxyphenyl 2,3-di-*O*-benzoyl-4-*O*-benzyl-β-D-glucopyranoside **195** (1.37 g, 2.34 mmol) was dried under vacuum for 1 h before being dissolved in anhydrous DCM (21 mL) under a nitrogen atmosphere. The resulting solution was cooled to 0 °C, then anhydrous pyridine (0.52 mL, 6.44 mmol) was added. Fluorenylmethyloxycarbonyl chloride (1.21 g, 4.69 mmol) was added in small portions over 30 min with stirring. Following complete fluorenylmethyloxycarbonyl chloride addition, the resulting solution was stirred for 2 h at RT, after which time TLC

(Hexane:EtOAc 7:3) showed the reaction to be complete. Methanol (0.28 mL) was added to quench excess fluorenylmethyloxycarbonyl chloride, then solvent was removed under reduced pressure, with co-evaporation with toluene (3 x 4 mL). The residue was dissolved in DCM (20 mL) and washed with water (2 x 15 mL), $NaHCO_{3\ (sat.\ aq.)}$ (15 mL) and brine (15 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. The crude material was purified by normal phase HPLC (Hexane:EtOAc) to give 4-methoxyphenyl 2,3-di-*O*-benzoyl-4-*O*-benzyl-6-*O*-(9-fluorenylmethoxycarbonyl)-D-glucopyranoside **196** (1.59 g, α:β = 1:1) as an anomeric mixture which was used directly in the next step. Fully protected glycoside **196** was dissolved in a mixture of MeCN:water (20 mL, 4:1) and cooled to 0 ℃. Ceric ammonium nitrate (2.16 g, 3.94 mmol) was added and the solution stirred for 45 min at 0 ℃, after which time TLC (Hexane:EtOAc 6:4) showed the reaction to be complete. Most of the solvent was removed under reduced pressure, and the residue was diluted with DCM (75 mL). The resulting solution was washed with water (2 x 70 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. Following purification by normal phase HPLC (Hexane:EtOAc) the free hemiacetal intermediate **197** (1.24 g, α:β = 4:1) was obtained as an anomeric mixture which was used directly in the next step. Hemiacetal **197** was dried for 1 h under vacuum before being dissolved in anhydrous DCM (18 mL) under a nitrogen atmosphere. To this solution trichloroacetonitrile (2.66 mL, 26.54 mmol) and sodium hydride (60 % in mineral oil, 0.0127 g, 0.531 mmol) were added and the resulting solution was stirred for 15 min at RT under a nitrogen atmosphere. After this time, TLC (Hexane:EtOAc 7:3) showed partial reaction of starting material to form two anomeric products. A further portion of NaH (0.0127 g, 0.531 mmol) was added and the solution was stirred for a further 30 min. After this time, TLC revealed a change in anomeric ratio as judged by spot intensity, with a greater proportion of α anomer than at 15 min. To continue anomerising product to bias the α anomer, the reaction mixture was heated to 40 ℃ for 2 h, then returned to RT and stirred for 16 h. After this time, TLC showed complete reaction of starting material with almost entirely α anomer present. Silica was added, then the solvent was removed under reduced pressure to dry load the product. Following column chromatography (Hexane:EtOAc 85:15), the title compound **191** was obtained as a white solid (1.11 g, 44 % over 4 steps); **^{1}H NMR** δ_H (400 MHz, Chloroform-*d*) 8.58 (1 H, s, NH), 8.02 – 7.96 (2 H, m, H_{arom}), 7.96 – 7.92 (2 H, m, H_{arom}), 7.79 (2 H, dd, *J* 7.5, 3.0, H_{arom}), 7.66 (2 H, m, H_{arom}), 7.57 – 7.46 (2 H, m, H_{arom}), 7.46 – 7.31 (8 H, m, H_{arom}), 7.17 (5

H, app q, *J* 3.5, 3.1, H_{arom}), 6.73 (1 H, d, *J* 3.6, H-1), 6.16 (1 H, td, *J* 9.7, 2.2, H-3), 5.49 (1 H, dd, *J* 10.2, 3.6, H-2), 4.67 – 4.58 (2 H, m, PhCH_2), 4.56 (1 H, dd, *J* 12.0, 2.1, H-6a), 4.52 – 4.40 (3 H, m, H-6b, OCH_2CH), 4.34 – 4.27 (2 H, m, H-5, OCH_2CH), 4.08 (1 H, t, *J* 9.7, H-4); **^{13}C NMR** δ_C (101 MHz, Chloroform-*d*) 165.72, 165.64 (2 C=O (Bz)), 160.80 (CNH), 154.97 (C=O (Fmoc)), 143.49, 143.34, 141.45, 136.76 (4˚ C_{arom}), 133.64, 133.52, 130.02, 129.85, 129.43, 128.72, 128.63, 128.60, 128.56, 128.54, 128.37, 128.35, 128.08, 127.38, 127.35, 125.34, 125.28, 120.23 (C_{arom}), 93.41 (C-1), 90.83 (CCl_3), 75.28 (PhCH_2), 75.10 (C-4), 72.35 (C-3), 71.66 (C-5), 70.87 (C-2), 70.31 (OCH_2CH), 65.63 (C-6), 46.87 (OCH_2CH); **m/z** (ESI-HRMS) $C_{44}H_{36}Cl_3O_{10}NNa^+$ ($[M + Na]^+$) calculated 866.1297; found 866.1289; **IR** ν_{max}/cm^{-1} 3344, 1726 (C=O), 1676, 1601, 1451, 1315, 1261, 1178, 1158, 1107, 1095, 1069, 1033, 1022, 1002, 971, 921, 826, 795, 759, 742, 709, 646; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{28}}$ + 53 [*c* 1.21, DCM].

4-Methoxyphenyl β-D-glucopyranoside 192

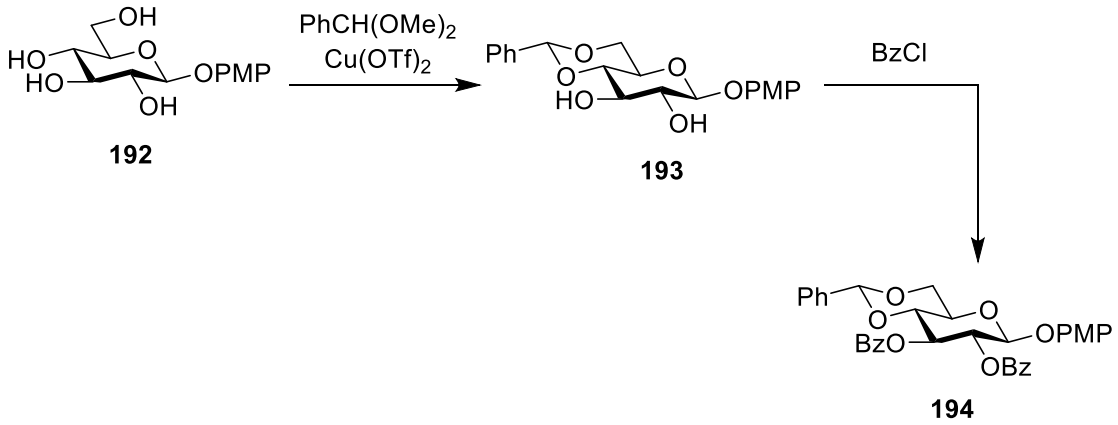


192

1,2,3,4,6-Penta-*O*-acetyl-β-D-glucopyranoside (20.00 g, 51.24 mmol) and 4-methoxyphenol (7.00 g, 56.36 mmol) were dried under vacuum for 1 h before being dissolved in anhydrous DCM (250 mL) and cooled to 0 ℃ under a nitrogen atmosphere. To this solution, boron trifluoride diethyl etherate (19.0 mL, 153.71 mmol) was added dropwise. The resulting solution was stirred at RT for 18 h. The mixture was then cautiously quenched using $NaHCO_{3\,(sat.\,aq.)}$ (400 mL). The product was extracted from the reaction mixture using DCM (100 mL) and the combined DCM phase was washed with water (200 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. The crude residue was dissolved in methanol (200 mL) at RT in air. Sodium methoxide (0.94 mL, 4.10 mmol, 25 % wt in MeOH) was added dropwise and the resulting solution was stirred for 2 h after which time TLC (DCM:MeOH 9:1) showed the reaction to be complete. The solution was then brought to pH 7, as monitored by universal indicator paper, using Amberlite acidic resin. The resulting neutralised solution was filtered, then silica was added. The solvent was removed under

reduced pressure to dry load the crude material on to silica. Following column chromatography (DCM:MeOH 9:1 → 8:2) the title compound **192** was obtained as a white solid (10.59 g, 72 % over 2 steps, α:β = 1:5.3) with spectroscopic details in accordance with the literature.[180] Note that only characteristic, non-overlapping, distinguishable NMR spectroscopy signals are given for the minor α anomer; **^{1}H NMR α anomer**: δ_H (400 MHz, Methanol-d_4) 5.33 (1 H, d, *J* 3.7, H-1), 3.54 (1 H, dd, *J* 9.8, 3.8, H-2); **β anomer**: δ_H (400 MHz, Methanol-d_4) 7.08 – 7.02 (2 H, m, H_{arom}), 6.86 – 6.80 (2 H, m, H_{arom}), 4.79 – 4.76 (1 H, m, H-1), 3.91 – 3.86 (1 H, m, H-6a), 3.74 (3 H, s, CH_3), 3.73 – 3.67 (1 H, m, H-6b), 3.47 – 3.37 (4 H, m, H-2, H-3, H-4, H-5); **^{13}C NMR α anomer**: δ_C (101 MHz, Methanol-d_4) 100.35 (H-1); **β anomer**: δ_C (101 MHz, Methanol-d_4) 156.61, 153.19 (2 4° C_{arom}), 119.20, 115.47 (C_{arom}), 103.41 (C-1), 78.04, 77.95, 74.94, 71.39 (C-2, C-3, C-4, C-5), 62.53 (C-6), 56.04 (CH_3); **m/z** (TLC-MS+ (ESI)) $C_{15}H_{21}NO_7Na^+$ ([M + MeCN + Na]$^+$) calculated 350; found 350; $C_{26}H_{36}O_{14}Na^+$ ([2M + Na]$^+$) calculated 595; found 595.

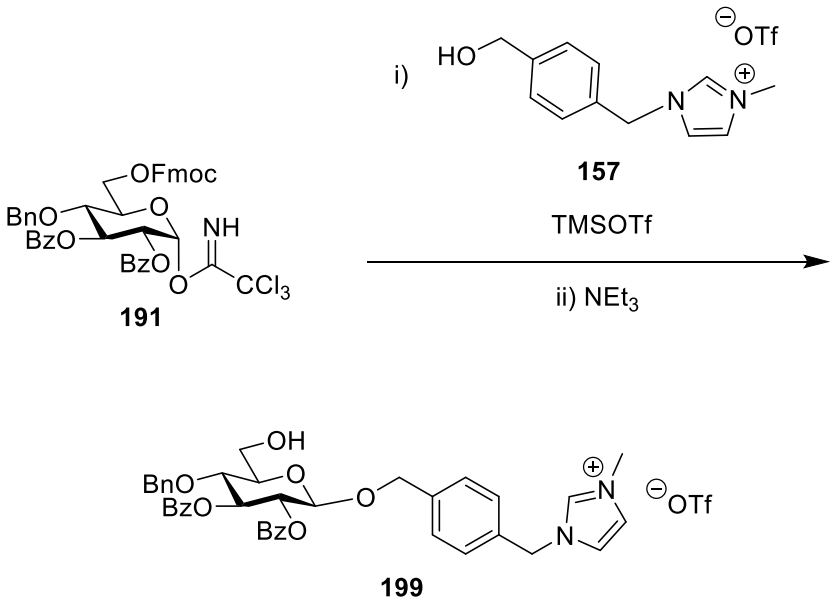
4-Methoxyphenyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside 194



4-Methoxyphenyl β-D-glucopyranoside **192** (10.59 g, 36.99 mmol) and copper^{II} triflate (0.669 g, 1.85 mmol) were dried under vacuum for 1 h. Anhydrous acetonitrile (370 mL) was added under a nitrogen atmosphere. Benzaldehyde dimethyl acetal (11.15 mL, 73.98 mmol) was added and the solution was sonicated under a nitrogen atmosphere for 90 min, after which time TLC (DCM:MeOH 9:1) and TLC-MS ($C_{22}H_{25}NO_7Na^+$ ([M + MeCN + Na]$^+$) calculated 438.2; found 438.2; $C_{40}H_{44}O_{14}Na^+$ ([2M + Na]$^+$) calculated 771.3; found 771.4) showed the reaction to be complete. The cloudy reaction mixture was quenched by addition of

triethylamine (5 mL). The solvent was removed under reduced pressure to give crude benzylidenated intermediate **193**. The crude intermediate **193** was dried under vacuum for 1 h, then dissolved in anhydrous pyridine (300 mL) under a nitrogen atmosphere. Benzoyl chloride (21.49 mL, 184.95 mmol) was added, and the solution was stirred at RT for 16 h under a nitrogen atmosphere. After this time, TLC (Hexane:EtOAc 8:2) showed the benzoylation reaction to be complete. Methanol (30 mL) was cautiously added to quench excess benzoyl chloride, then solvent was removed under reduced pressure. The residue was dissolved in DCM (200 mL) and the resulting organic solution was washed with 1 M $HCl_{(aq.)}$ (2 x 150 mL), $NaHCO_{3\ (sat.\ aq.)}$ (150 mL) and water (150 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. Following purification by column chromatography (Hexane:EtOAc 1:0 → 85:15 → 8:2 → 7:3 → EtOAc) the title compound **194** was obtained as an off-white solid (15.01 g, 70 % over 2 steps); **^{1}H NMR** δ H (500 MHz, Chloroform-d) 7.99 (4 H, ddd, J 8.5, 4.9, 1.4, H_{arom}), 7.55 – 7.48 (2 H, m, H_{arom}), 7.45 – 7.30 (9 H, m, H_{arom}), 6.96 – 6.91 (2 H, m, H_{arom} (PMP)), 6.81 – 6.76 (2 H, m, H_{arom} (PMP)), 5.85 (1 H, t, J 9.5, H-3), 5.72 (1 H, dd, J 9.4, 7.7, H-2), 5.58 (1 H, s, PhCHOO), 5.26 (1 H, d, J 7.7, H-1), 4.47 (1 H, dd, J 10.6, 5.0, H-6a), 4.04 (1 H, t, J 9.5, H-4), 3.94 (1 H, t, J 10.3, H-6b), 3.80 (1 H, td, J 9.7, 4.9, H-5), 3.75 (3 H, s, CH_3); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) 165.74, 165.35 (2 C=O), 156.00, 151.12 (2 4˚ C_{arom}), 136.84, 133.44, 133.29, 129.99, 129.96, 129.50, 129.31, 129.21, 128.55, 128.47, 128.35, 126.27 (C_{arom}), 119.15, 114.71 (C_{arom} (PMP)), 101.70 (PhCHOO), 101.59 (C-1), 78.76 (C-4), 72.55 (C-2), 72.19 (C-3), 68.78 (C-6), 66.91 (C-5), 55.75 (CH_3); **m/z** (ESI-HRMS) $C_{34}H_{34}NO_9^+$ ([M + NH_4]$^+$) calculated 600.2228; found 600.2220; $C_{27}H_{23}O_7^+$ ([M - OPMP]$^+$) calculated 459.1438; found 459.1432; **IR** ν_{max}/cm^{-1} 1725 (C=O), 1601, 1506, 1451, 1374, 1314, 1270, 1216, 1179, 1092s, 1070s, 1027, 1008, 912, 851, 829, 756, 708s, 650; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{24}}$ + 41 [*c* 0.90, DCM].

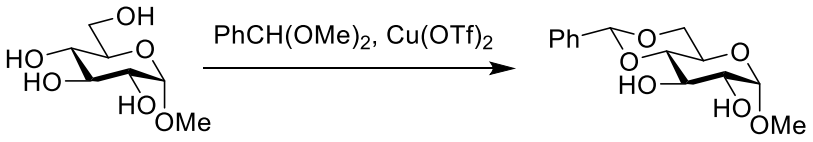
4-(1-methyl-3-methyleneimidazolium)benzyl 2,3-di-*O*-benzoyl-4-*O*-benzyl-β-D-glucopyranoside trifluoromethanesulfonate 199



Following the general procedure for I-Tag glycosylations in flow using glycosyl acceptor 1-(4-(hydroxymethyl)benzyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate **157** (0.0282 g, 0.080 mmol, 1 eq) and glycosyl donor 2,3-di-*O*-benzoyl-4-*O*-benzyl-6-*O*-(9-fluorenylmethoxycarbonyl)-α-D-glucopyranosyl trichloroacetimidate **191** (0.1352 g, 0.160 mmol, 2 eq). 0.40 mL of anhydrous MeCN was added to the donor/acceptor vial, resulting in a solution of volume 0.49 mL and therefore approximately 0.163 M in acceptor and 0.327 M in donor. To the other vial, 0.80 mL of anhydrous acetonitrile was added, followed by trimethylsilyl trifluoromethanesulfonate (8.7 µL, 0.048 mmol) to make a 0.06 M solution. The flow reaction was performed at RT. Reaction solution was collected into a mixture of DCM:NEt_3 3:1 (4 mL) for a total of 6 min 10 sec and the resulting solution was stirred at RT for 20 min in air to remove the Fmoc group. After this time TLC-MS showed complete conversion to the desired product. The solution was diluted with DCM (5 mL) and washed with 1 M $HCl_{(aq.)}$ (8 mL) and water (8 mL). The combined aqueous phases were washed with a portion of DCM (5 mL), then DCM washes were combined and the aqueous washes discarded. The DCM phase was then washed with $NaHCO_{3\,(sat.\ aq.)}$ (8 mL) and water (8 mL). The combined aqueous phases were washed with a portion of DCM (5 mL), then DCM washes were combined and the aqueous washes discarded. The DCM phase was dried using magnesium sulfate, filtered and the solvent was removed under

reduced pressure. The crude residue was washed with hexane:Et_2O 1:1 (2 x 6 mL), hexane:Et_2O 2:3 (2 x 6 mL) and hexane:Et_2O 1:2 (6 mL) then dried under reduced pressure to yield the title compound **199** as a solid (0.0394 g, 74 %); **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 9.02 (1 H, s, NCHN), 7.88 (4 H, app t, *J* 8.8, H_{arom}), 7.47 (2 H, app t, *J* 7.4, H_{arom}), 7.37 – 7.29 (4 H, m, H_{arom}), 7.23 (1 H, s, NC*H*CHN), 7.18 – 7.09 (10 H, m, H_{arom}, NCHC*H*N), 5.69 (1 H, t, *J* 9.6, H-3), 5.35 (1 H, dd, *J* 9.9, 8.0, H-2), 5.19 (2 H, s, NCH_2), 4.85 – 4.74 (2 H, m, H-1, (C-1)OC*H*H), 4.63 – 4.53 (3 H, m, (C-4)OC*H*$_2$Ph, (C-1)OCH*H*), 4.00 – 3.91 (2 H, m, H-4, H-6a), 3.85 – 3.80 (1 H, m, H-6b), 3.79 (3 H, s, NCH_3), 3.58 (1 H, dt, *J* 9.5, 3.2, H-5); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) 165.79, 165.54 (2 C=O), 138.58, 137.32 (4˚ C_{arom}), 136.64 (NCHN), 133.47, 133.34, 132.50, 129.75, 129.39, 129.27, 128.90, 128.53, 128.49, 128.41, 128.25, 128.00 (C_{arom}), 123.81 (N*C*HCHN), 122.11 (NCH*C*HN), 100.39 (C-1), 75.83 (C-5), 75.70 (C-4), 75.04 (C-3), 74.87 ((C-4)O*C*H$_2$Ph), 72.29 (C-2), 70.63 ((C-1)O*C*H$_2$), 61.25 (C-6), 52.91 (NCH_2), 36.33 (NCH_3); **m/z** (ESI-HRMS) $C_{39}H_{39}N_2O_8^+$ ([M - OTf]$^+$) calculated 663.2701; found 663.2695; (TLC-MS- (ESI)) $CF_3O_3S^-$ ([OTf]$^-$) calculated 148.9; found 149.1; **IR** ν_{max}/cm^{-1} 3470br (OH), 3152w, 3066w, 2926w, 1723 (C=O), 1601, 1583, 1574, 1563, 1452, 1411w, 1362w, 1315, 1255s, 1225, 1157, 1095, 1070s, 1047, 1028s, 1001, 911, 853, 826, 804, 755, 736, 711, 638, 623, 574, 518; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{24}}$ + 11 [*c* 0.70, DCM]. Note that due to sparing solubility of the product in DCM, the solution used to determine optical rotation was passed through a syringe tip filter prior to the measurement being taken.

Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside 204

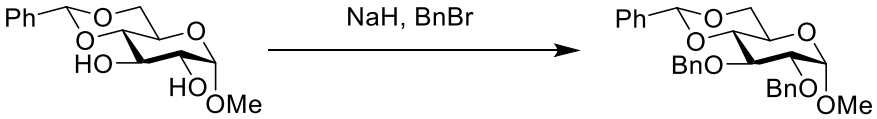


115 **204**

Methyl α-D-glucopyranoside **115** (10.00 g, 51.50 mmol) was dried under vacuum for 1 h in a flame-dried flask before being dissolved in anhydrous acetonitrile (200 mL) under a nitrogen atmosphere. Copper^{II} triflate (0.93 g, 2.57 mmol) and benzaldehyde dimethyl acetal (9.3 mL, 61.80 mmol) were added and the solution was sonicated under a nitrogen atmosphere for 3 h, after which time the reaction was quenched by addition of triethylamine (2 mL) and the solvent was removed under reduced pressure. Following purification by

column chromatography (DCM → DCM:MeOH 96:4) the title compound **204** was obtained as a white solid (13.13 g, 90 %), with spectroscopic details in accordance with the literature;[181] **^{1}H NMR** δ_H (400 MHz, Chloroform-*d*) 7.53 – 7.45 (2 H, m, H_{arom}), 7.42 – 7.33 (3 H, m, H_{arom}), 5.52 (1 H, s, PhC(*H*)OO), 4.76 (1 H, d, *J* 3.7, H-1), 4.28 (1 H, dd, *J* 9.6, 4.2, H-6a), 3.91 (1 H, t, *J* 9.2, H-3), 3.84 – 3.68 (2 H, m, H-5, H-6b), 3.60 (1 H, t, *J* 6.0, H-2), 3.48 (1 H, at, *J* 9.2, H-4), 3.44 (3 H, s, OCH_3); **^{13}C NMR** δ_C (101 MHz, Chloroform-*d*) 137.17 (4˚ C_{arom}), 129.37, 128.45, 126.45 (C_{arom}), 102.06 (Ph*C*(H)OO), 99.93 (C-1), 81.06 (C-4), 72.96 (C-2), 71.77 (C-3), 69.05 (C-6), 62.49 (C-5), 55.67 (OCH_3); **m/z** (ESI-MS+) $C_{14}H_{18}O_6Na^+$ ([M + Na]$^+$) calculated: 305.1; found 305.1; $C_{14}H_{19}O_6^+$ ([M + H]$^+$) calculated 283.1; found 283.1.

Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside 205

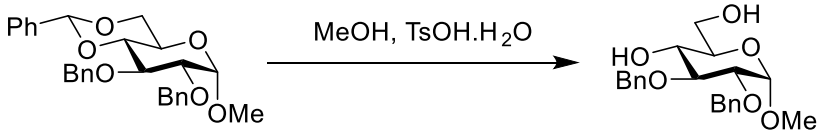


204 **205**

Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside **204** (1.10 g, 3.90 mmol) was dried under vacuum for 1 h in a flame-dried flask before being dissolved in anhydrous DMF (28 mL) under a nitrogen atmosphere. The solution was cooled to 0 ℃ and sodium hydride (60 % in mineral oil, 0.39 g, 9.75 mmol) was added before the reaction mixture was stirred for 15 min at 0 ℃ then 30 min at RT. The solution was cooled to 0 ℃ again and benzyl bromide (1.39 mL, 11.70 mmol) was added dropwise. After being left to stir under nitrogen overnight, methanol (1 mL) was added to the reaction mixture then solvent was removed under reduced pressure. The residue was dissolved in DCM (35 mL), washed with water (2 x 12 mL) and brine (12 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure to give a crude product. Following purification by column chromatography (Hexane:EtOAc 4:1) the title compound **205** was obtained as a white solid (1.16 g, 64 %) with spectroscopic details in accordance with the literature;[108] **^{1}H NMR** δ_H (400 MHz, Chloroform-*d*) 7.50 (2 H, dd, *J* 7.4, 2.2, H_{arom}), 7.42 – 7.27 (13 H, m, H_{arom}), 5.56 (1 H, s, PhC(*H*)OO), 4.92 (1 H, d, *J* 11.3, PhC*H*H), 4.86 (1 H, d, *J* 12.1, PhC*H*H), 4.84 (1 H, d, *J* 11.3, PhCH*H*), 4.71 (1 H, d, *J* 12.1, PhCH*H*), 4.60 (1 H, d, *J* 3.7, H-1), 4.27 (1 H, dd, *J* 10.1, 4.7, H-6a), 4.05 (1 H, t, *J* 9.3, H-3), 3.84 (1 H, td, *J* 9.9, 4.7, H-5), 3.71 (1 H, t, *J* 10.2, H-6b), 3.61 (1 H, t, *J* 9.3, H-4), 3.57 (1 H, dd, *J* 9.3, 3.8, H-2),

3.41 (3 H, s, OCH_3); **^{13}C NMR** δ_C (101 MHz, Chloroform-*d*) 138.85, 138.29, 137.54 (3 4° C_{arom}), 129.04, 128.58, 128.44, 128.35, 128.26, 128.16, 128.05, 127.72, 126.16 (15 C_{arom}), 101.39 (Ph*C*(H)OO), 99.38 (C-1), 82.27 (C-4), 79.30 (C-2), 78.74 (C-3), 75.49 (Ph*C*H_2), 73.93 (Ph*C*H_2), 69.20 (C-6), 62.46 (C-5), 55.49 (OCH_3); **m/z** (ESI-MS+) $C_{28}H_{30}O_6Na^+$ ([M + Na]$^+$) calculated 485.19; found 485.19.

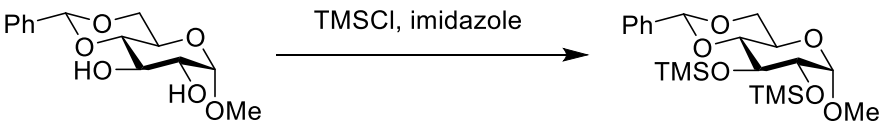
Methyl 2,3-di-*O*-benzyl-α-D-glucopyranoside 206



205 **206**

Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside **205** (1.05 g, 2.27 mmol), methanol (11 mL) and *p*-toluenesulfonic acid monohydrate (0.043 g, 0.23 mmol) were added to a flask and sonicated for 1 h at RT, after which time triethylamine (0.42 mL) was added and the solvent was removed under reduced pressure. Following purification by column chromatography (Hexane:EtOAc 3:1 → 1:1 → EtOAc) the title compound **206** was obtained as a yellow oil (0.79 g, 93 %) with spectroscopic details in accordance with the literature;[108] **^{1}H NMR** δ_H (400 MHz, Chloroform-*d*) 7.42 – 7.28 (10 H, m, H_{arom}), 5.01 (1 H, d, *J* 11.4, PhC*H*H), 4.76 (2 H, dd, *J* 11.8, 1.9, PhCH_2), 4.66 (1 H, d, *J* 12.0, PhCH*H*), 4.62 (1 H, d, *J* 3.6, H-1), 3.82 (1 H, at, *J* 8.9, H-3), 3.76 (2 H, app bs, H-6a, H-6b), 3.64 – 3.54 (2 H, m, H-4, H-5), 3.50 (1 H, dd, *J* 9.5, 3.5, H-2), 3.38 (3 H, s, OCH_3); **^{13}C NMR** δ_C (101 MHz, Chloroform-*d*) 138.76, 138.03 (2 4° C_{arom}), 128.54, 128.47, 128.2 , 128.09, 127.96, 127.81, 126.69 (10 C_{arom}), 98.18 (C-1), 81.41 (C-3), 79.78 (C-2), 75.42 (Ph*C*H_2), 73.14 (Ph*C*H_2), 70.88 (C-5), 70.25 (C-4), 62.10 (C-6), 55.23 (OCH_3); **m/z** (ESI-MS+) $C_{21}H_{26}O_6Na^+$ ([M + Na]$^+$) calculated 397.16; found 397.16.

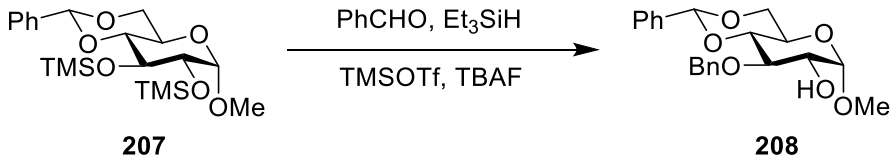
Methyl 2,3-di-*O*-trimethylsilyl-4,6-*O*-benzylidene-α-D-glucopyranoside 207



204 **207**

Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside **204** (6.00 g, 21.25 mmol) was dried under vacuum in a flame-dried flask for 1 h, before being dissolved in anhydrous DCM (200 mL) under a nitrogen atmosphere. Imidazole (5.79 g, 85.00 mmol) and TMSCl (8.1 mL, 63.75 mmol) were added and the resulting solution was stirred for 24 h at RT. After this time, the solution was washed with water (2 x 150 mL) and brine (100 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. Following purification by column chromatography (Hexane:EtOAc, 99:1 → 96:4) the title compound **207** was obtained as a white solid (7.33 g, 81 %) with spectroscopic details in accordance with the literature;[108] **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 7.49 (2 H, dd, *J* 7.6, 2.0, H_{arom}), 7.39 – 7.31 (3 H, m, H_{arom}), 5.50 (1 H, s, PhC(*H*)OO), 4.62 (1 H, d, *J* 3.7, H-1), 4.27 (1 H, dd, *J* 10.0, 4.8, H-6a), 3.96 (1 H, t, *J* 8.9, H-3), 3.81 (1 H, td, *J* 9.9, 4.8, H-5), 3.71 (1 H, t, *J* 10.2, H-6b), 3.62 (1 H, dd, *J* 8.8, 3.8, H-2), 3.42 (3 H, s, OCH_3), 3.41 (1 H, t, *J* 9.3, H-4), 0.17 (9 H, s, $Si(CH_3)_3$), 0.10 (9 H, s, $Si(CH_3)_3$); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) 137.56 (4° C_{arom}), 129.01, 128.25, 126.32 (5 C_{arom}), 101.84 (Ph*C*(H)OO), 101.16 (C-1), 82.26 (C-4), 74.30 (C-2), 71.92 (C-3), 69.26 (C-6), 62.48 (C-5), 55.52 (OCH_3), 0.82 ($Si(CH_3)_3$), 0.47 ($Si(CH_3)_3$); **m/z** (ESI-MS+) $C_{20}H_{34}O_6Si_2Na^+$ ([M + Na]$^+$) calculated 449.18; found 449.18.

Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside 208

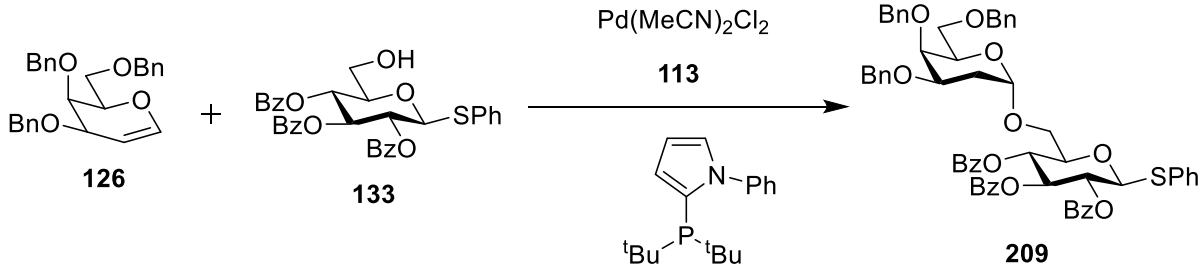


207 **208**

Methyl 2,3-di-*O*-trimethylsilyl-4,6-*O*-benzylidene-α-D-glucopyranoside **207** (7.30 g, 17.11 mmol), was added to a flask containing activated molecular sieves, before being dissolved in anhydrous DCM (150 mL) under a nitrogen atmosphere. Benzaldehyde (2.08 mL, 20.53 mmol) and triethylsilane (3.28 mL, 20.53 mmol) were added and the solution was stirred for 30 min at RT. The solution was then cooled to -78 °C and trimethylsilyl trifluoromethanesulfonate (0.31 mL, 1.71 mmol) was added dropwise. The reaction was monitored by TLC. After 1 h, TBAF (1.0 M in THF, 34.2 mL, 34.22 mmol) was added and the solution was gradually warmed to RT. After 90 min, the reaction mixture was passed through a Celite pad and the solvent was removed under reduced pressure. Following purification by

column chromatography (Hexane:EtOAc, 7:3 → 6:4) the title compound **208** was obtained as a white solid (3.08 g, 48 %) with spectroscopic details in accordance with the literature;[108] **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 7.54 – 7.47 (2 H, m, H_{arom}), 7.39 (5 H, m, H_{arom}), 7.36 – 7.27 (3 H, m, H_{arom}), 5.58 (1 H, s, PhC(*H*)OO), 4.97 (1 H, d, *J* 11.6, PhC*H*H), 4.81 (1 H, d, *J* 3.5, H-1), 4.80 (1 H, d, *J* 12.0, PhCH*H*), 4.31 (1 H, dd, *J* 10.0, 4.6, H-6a), 3.88 – 3.81 (2 H, m, H-3, H-5), 3.78 (1 H, d, *J* 10.2, H-6b), 3.76 – 3.71 (1 H, m, H-2), 3.65 (1 H, t, *J* 9.2, H-4), 3.45 (3 H, s, OCH_3), 2.38 (1 H, d, *J* 7.4, OH); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) 138.57, 137.46 (2 4° C_{arom}), 129.07, 128.50, 128.35, 128.10, 127.82, 126.14 (C_{arom}), 101.40 (Ph*C*(H)OO), 99.99 (C-1), 82.06 (C-4), 78.96 (C-3), 74.91 (Ph*C*H_2), 72.52 (C-2), 69.13 (C-6), 62.70 (C-5), 55.51 (OCH_3); **m/z** (ESI-MS+) $C_{21}H_{24}O_6Na^+$ ([M + Na]$^+$) calculated 395.1; found 395.1.

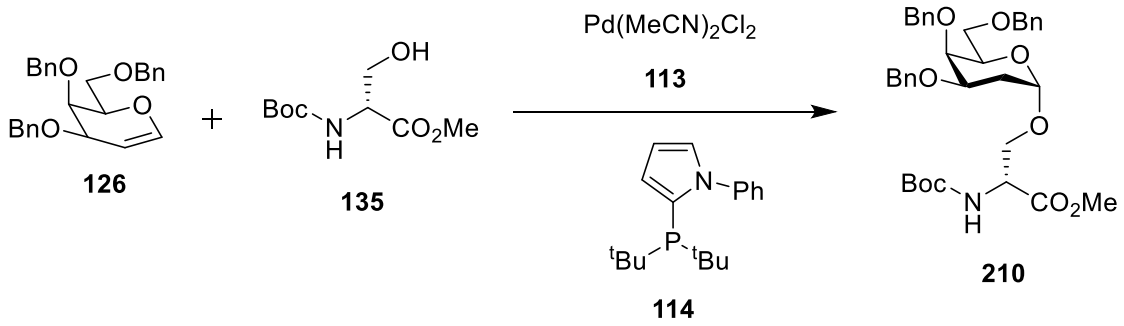
Phenyl 2,3,4-tri-*O*-benzoyl-6-*O*-(3,4,6-tri-*O*-benzyl-α-D-lyxo-hexapyranosyl)-β-D-thioglucopyranoside 209



Following the general procedure for palladium catalysed glycosylation reactions, glycosyl donor tri-*O*-benzyl-D-galactal **126** (0.050 g, 0.120 mmol), glycosyl acceptor phenyl 2,3,4-tri-*O*-benzoyl-β-D-thioglucopyranoside **133** (0.053 g, 0.090 mmol), metal catalyst bis(acetonitrile)dichloropalladium (II) **113** (0.009 g, 0.036 mmol) and ligand 2-(di-*tert*-butylphosphanyl)-1-phenyl-1H-pyrrole **114** (0.010 g, 0.036 mmol) were dissolved in 1 mL of anhydrous DCM. The reaction mixture was stirred for 21 h before being quenched. Following purification by column chromatography (Hexane:EtOAc 9:1 → 8:2), during which α and β anomers were separated, the title compound **209** was obtained as a solid (0.051 g, 56 %) with spectroscopic details in accordance with the literature;[108] **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 8.00 – 7.93 (2 H, m, H_{arom}), 7.93 – 7.87 (2 H, m, H_{arom}), 7.83 – 7.77 (2 H, m, H_{arom}), 7.56 – 7.19 (29 H, m, H_{arom}), 5.86 (1 H, t, *J* 9.6, H-3), 5.56 (1 H, t, *J* 9.8, H-4), 5.46 (1 H, t, *J* 9.7, H-2), 5.00 (1 H, d, *J* 10.0, H-1), 4.99 (2 H, app d, *J* 3.0, H-1'), 4.88 (1 H, d, *J* 11.6, PhC*H*H), 4.57 (1 H,

d, *J* 11.6, PhC*H*H), 4.49 (2 H, s, PhCH_2), 4.38 (1 H, d, *J* 11.9, PhC*H*H), 4.31 (1 H, d, *J* 12.0, PhC*H*H), 4.01 (1 H, ddd, *J* 9.9, 4.9, 3.2, H-5), 3.92 – 3.81 (3 H, m, H-3', H-4', H-6a), 3.81 (1 H, s, H-4'), 3.67 (1 H, dd, *J* 11.2, 3.3, H-6b), 3.46 (2 H, dd, *J* 6.5, 2.2, H-6a', H-6b'), 2.16 (1 H, td, *J* 12.4, 3.7, H-2ax'), 1.97 – 1.89 (1 H, ddt, *J* 12.7, 4.6, 1.3, H-2eq'); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) 165.96, 165.23, 165.21 (3 C=O) , 139.03, 138.76, 138.37 (3 4° C_{arom}(Bn)), 133.48, 133.43, 133.30 (3 4° C_{arom}(Bz)), 133.02 (C_{arom}), 132.19 (4° C_{arom}(SPh)), 130.01, 129.90, 129.87, 129.41, 129.20, 129.05, 129.03, 128.55, 128.52, 128.44, 128.40, 128.35, 128.32, 128.30, 127.70, 127.62, 127.58, 127.47 (C_{arom}), 98.42 (C-1'), 86.16 (C-1), 77.15 (C-5), 74.91 (C-3'), 74.57 (C-3), 74.40 (Ph*C*H_2), 73.32 (Ph*C*H_2), 73.19 (C-4'), 70.67, 70.64 (Ph*C*H_2, C-2), 69.98 (C-5'), 69.79 (C-4), 69.62 (C-6'), 66.55 (C-6), 31.04 (C-2'); **m/z** (ESI-MS+) $C_{60}H_{56}O_{12}SNa^+$ ([M + Na]$^+$) calculated 1023.3; found 1023.3.

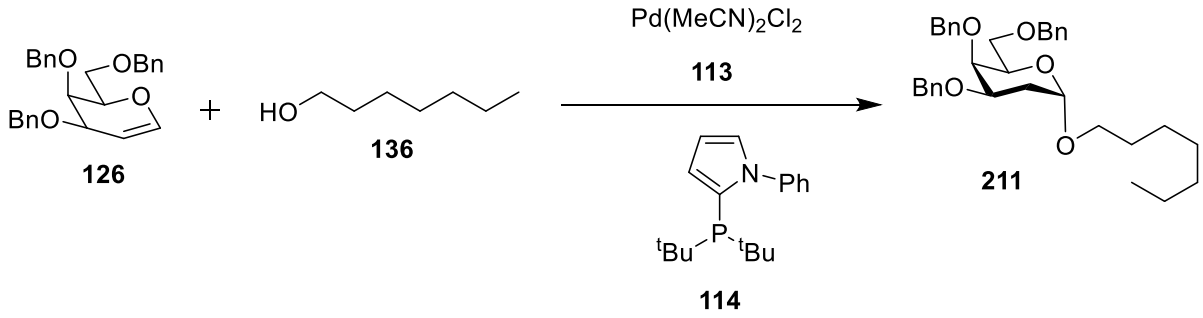
2-Deoxy-3,4,6-tri-*O*-benzyl-α-D-lyxo-hexopyranosyl-(1→O)-*N*-*tert*-butoxycarbonyl-L-serine methyl ester 210



Following the general procedure for palladium catalysed glycosylation reactions, glycosyl donor tri-*O*-benzyl-D-galactal **126** (0.100 g, 0.240 mmol), glycosyl acceptor Boc-L-serine methyl ester (0.039 g, 0.180 mmol), metal catalyst bis(acetonitrile)dichloropalladium (II) **113** (0.019 g, 0.072 mmol) and ligand 2-(di-*tert*-butylphosphanyl)-1-phenyl-1H-pyrrole **114** (0.021 g, 0.072 mmol) were dissolved in 2 mL of anhydrous DCM. The reaction mixture was stirred for 18 h before being quenched. Following purification by column chromatography (Hexane:EtOAc 7:3) the title compound **210** was obtained as a colourless oil (0.101 g, 88 %), with spectroscopic details in accordance with the literature;[182] **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 7.40 – 7.20 (15 H, m, H_{arom}), 5.45 (1 H, d, *J* 8.9, NH), 4.93 (1 H, bs, H-1), 4.92 (1 H, d, *J* 11.5, PhC*H*H), 4.64 – 4.55 (3 H, m, PhCH_2), 4.52 (1 H, d, *J* 11.8, PhC*H*H), 4.49 – 4.45 (1 H, m, NHC*H*), 4.43 (1 H, d, *J* 11.8, PhCH*H*), 3.93 (1 H, bs, H-4), 3.92 – 3.80 (4 H, m, H-3, H-5,

NHCHC*H*$_2$O), 3.73 (3 H, s, OCH$_3$), 3.63 – 3.54 (2 H, m, H-6a, H-6b), 2.21 (1 H, td, *J* 12.4, 3.8, H-2ax), 1.94 (1 H, dd, *J* 12.8, 4.3, H-2eq), 1.45 (9 H, s, OC(CH$_3$)$_3$); **^{13}C NMR** δ $_C$ (126 MHz, Chloroform-*d*) 171.24 (*C*(O)OCH$_3$), 155.62 (NH*C*(O)), 138.90, 138.49, 138.15 (3 4° C$_{arom}$), 128.54, 128.51, 128.34, 128.33, 127.93, 127.81, 127.71, 127.66, 127.53 (15 C$_{arom}$), 99.01 (C-1), 80.17 (O*C*(CH$_3$)$_3$), 74.48, 74.44 (C-3, Ph*C*H$_2$), 73.60 (Ph*C*H$_2$), 72.84 (C-4), 70.56 (Ph*C*H$_2$), 70.38 (C-5), 69.33 (C-6), 68.70 (NHCH*C*H$_2$O), 54.13 (NH*C*H), 52.55 (OCH$_3$), 31.13 (C-2), 28.46 (3 OC(*C*H$_3$)$_3$); **m/z** (ESI-MS+) $C_{36}H_{45}NO_9Na^+$ ([M + Na]$^+$) calculated 658.3; found 658.3.

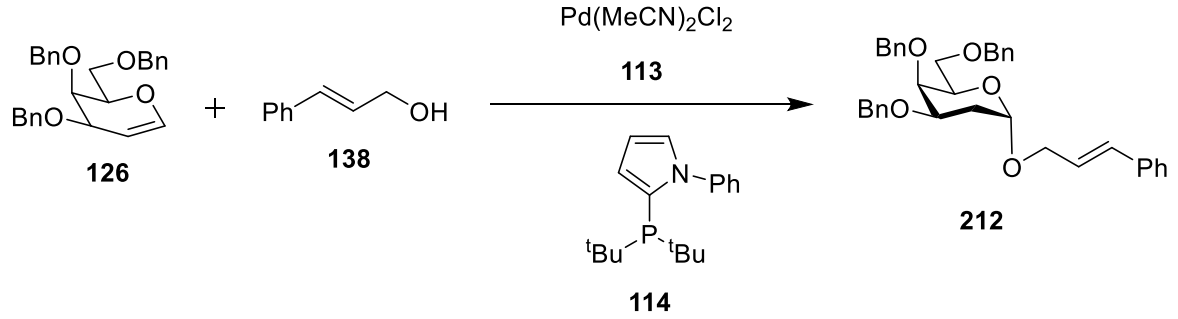
Heptyl 3,4,6-tri-*O*-benzyl-α-D-lyxo-hexapyranoside 211



Following the general procedure for palladium catalysed glycosylation reactions, glycosyl donor tri-*O*-benzyl-D-galactal **126** (0.100 g, 0.240 mmol), 1-heptanol **136** (0.021 g, 0.180 mmol), metal catalyst bis(acetonitrile)dichloropalladium (II) **113** (0.019 g, 0.072 mmol) and ligand 2-(di-*tert*-butylphosphanyl)-1-phenyl-1H-pyrrole **114** (0.021 g, 0.072 mmol) were dissolved in 2 mL of anhydrous DCM. The reaction mixture was stirred for 18.5 h before being quenched. Following purification by column chromatography (Hexane:EtOAc 9:1) the title compound **211** was obtained as a yellow oil (0.060 g, 63 %, α:β = 9:1); **^{1}H NMR** δ $_H$ (500 MHz, Chloroform-*d*) 7.38 – 7.27 (15 H, m, H$_{arom}$), 4.99 (1 H, app d, *J* 3.5, H-1), 4.95 (1 H, d, *J* 11.7, PhC*H*H), 4.64 (1 H, d, *J* 11.5, PhCH*H*), 4.62 (2 H, s, PhC*H*$_2$), 4.53 (1 H, d, *J* 11.8, PhC*H*H), 4.45 (1 H, d, *J* 11.9, PhCH*H*), 3.96 (1 H, ddd, *J* 11.6, 4.6, 2.5, H-3), 3.94 (1 H, d, *J* 3.2, H-4), 3.92 (1 H, t, *J* 6.5, H-5), 3.66 – 3.56 (3 H, m, H-6a, H-6b, OC*H*H(CH$_2$)$_5$CH$_3$), 3.38 (1 H, dt, *J* 9.7, 6.6, OCH*H*(CH$_2$)$_5$CH$_3$), 2.24 (1 H, td, *J* 12.3, 11.7, 4.0, H-2ax), 2.03 – 1.98 (1 H, m, H-2eq), 1.57 (2 H, p, *J* 6.7, OCH$_2$C*H*$_2$(CH$_2$)$_4$CH$_3$), 1.35 – 1.23 (8 H, m, O(CH$_2$)$_2$(C*H*$_2$)$_4$CH$_3$), 0.93 – 0.88 (3 H, m, O(CH$_2$)$_6$C*H*$_3$); **^{13}C NMR** δ $_C$ (126 MHz, Chloroform-*d*) 139.09, 138.75, 138.31 (3 4° C$_{arom}$), 128.51, 128.50, 128.36, 128.33, 127.88, 127.77, 127.61, 127.60, 127.46 (15 C$_{arom}$), 97.86 (C-1), 75.09 (C-3), 74.41 (Ph*C*H$_2$), 73.60 (Ph*C*H$_2$), 73.22 (C-4), 70.60 (Ph*C*H$_2$), 69.93 (C-5), 69.77 (C-6), 67.65 (O*C*H$_2$(CH$_2$)$_5$CH$_3$), 31.97 (O(CH$_2$)$_2$(*C*H$_2$)$_4$CH$_3$), 31.46 (C-2), 29.71 (OCH$_2$*C*H$_2$(CH$_2$)$_4$CH$_3$), 29.27,

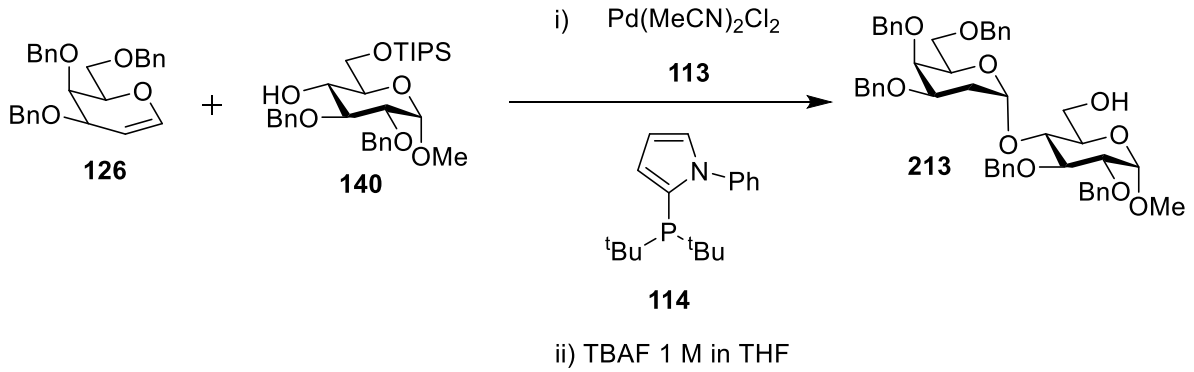
26.35, 22.77 (3 $O(CH_2)_2(CH_2)_4CH_3$), 14.25 ($O(CH_2)_6CH_3$); **m/z** (ESI-HRMS) $C_{34}H_{44}O_5Na^+$ ([M + Na]$^+$) calculated 555.3081; found 555.3076; **IR** ν_{max}/cm^{-1} 3030, 2924, 2856, 1725, 1496, 1454, 1357, 1273, 1204, 1165, 1094, 1057, 1027, 903, 734, 697.

Cinnamyl 3,4,6-tri-*O*-benzyl-α-D-lyxo-hexapyranoside 212



Following the general procedure for palladium catalysed glycosylation reactions, glycosyl donor tri-*O*-benzyl-D-galactal **126** (0.100 g, 0.240 mmol), cinnamyl alcohol **138** (0.024 g, 0.180 mmol), metal catalyst bis(acetonitrile)dichloropalladium (II) **113** (0.019 g, 0.072 mmol) and ligand 2-(di-*tert*-butylphosphanyl)-1-phenyl-1H-pyrrole **114** (0.021 g, 0.072 mmol) were dissolved in 2 mL of anhydrous DCM. The reaction mixture was stirred for 23 h before being quenched. Following purification by column chromatography (Hexane:EtOAc 11:1) the title compound **212** was obtained as a solid (0.065 g, 66 %), with spectroscopic details in accordance with the literature;[183] **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 7.40 – 7.20 (20 H, m, H_{arom}), 6.57 (1 H, d, *J* 15.9, OCH_2CH=C*H*Ph), 6.29 (1 H, dt, *J* 15.9, 6.2, OCH_2CH=CHPh), 5.10 (1 H, app d, *J* 3.5, H-1), 4.94 (1 H, d, *J* 11.6, PhC*H*HO), 4.63 (3 H, d, *J* 11.9, PhCH*H*O, PhCH_2O), 4.52 (1 H, d, *J* 11.8, PhC*H*HO), 4.43 (1 H, d, *J* 11.8, PhCH*H*O), 4.29 (1 H, ddd, *J* 12.9, 5.7, 1.5, OC*H*HCH=CHPh), 4.13 (1 H, ddd, *J* 12.9, 6.7, 1.3, OCH*H*CH=CHPh), 4.01 – 3.95 (2 H, m, H-3, H-5), 3.94 (1 H, bs, H-4), 3.61 (2 H, dd, *J* 6.4, 3.4, H-6a, H-6b), 2.27 (1 H, td, *J* 12.4, 3.8, H-2ax), 2.05 (1 H, dd, *J* 12.7, 4.7, H-2eq); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) 139.04, 138.70, 138.28 (3 4° C_{arom}(Bn)), 136.82 (4° C_{arom}CH=CH), 132.83 (OCH_2CH=*C*HPh), 128.68, 128.55, 128.52, 128.36, 128.35, 127.90, 127.83, 127.81, 127.66, 127.63, 127.45, 126.64 (16 C_{arom}), 125.73 (OCH_2*C*H=CHPh), 97.30 (C-1), 74.98 (C-3), 74.44 (Ph*C*H_2), 73.64 (Ph*C*H_2), 73.21 (C-4), 70.63 (Ph*C*H_2), 70.19 (C-5), 69.84 (C-6), 67.82 (O*C*H_2CH=CHPh), 31.34 (C-2); **m/z** (ESI-MS+) $C_{36}H_{38}O_5Na^+$ ([M + Na]$^+$) calculated 573.3; found 573.3.

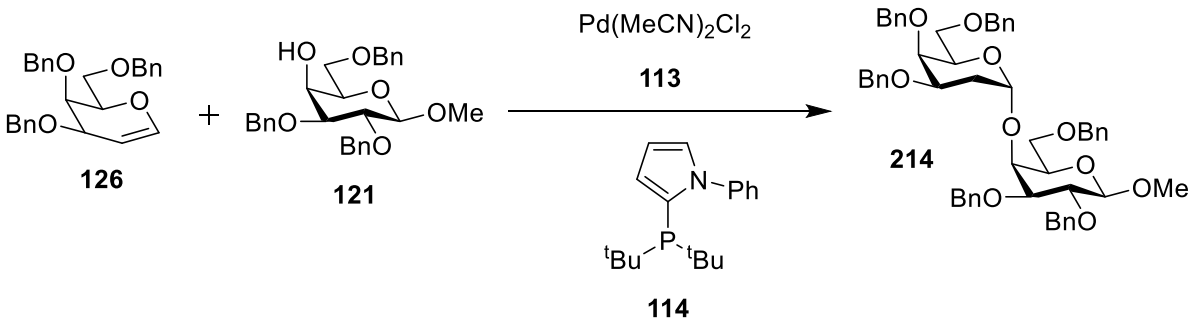
Methyl 2,3-di-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-benzyl-α-D-lyxo-hexapyranosyl)-α-D-glucopyranoside 213



Following the general procedure for palladium catalysed glycosylation reactions, glycosyl donor tri-*O*-benzyl-D-galactal **126** (0.100 g, 0.240 mmol), glycosyl acceptor methyl 2,3-di-*O*-benzyl-6-*O*-triisopropylsilyl-α-D-glucopyranoside **140** (0.096 g, 0.180 mmol), metal catalyst bis(acetonitrile)dichloropalladium (II) **113** (0.019 g, 0.072 mmol) and ligand 2-(di-*tert*-butylphosphanyl)-1-phenyl-1H-pyrrole **114** (0.021 g, 0.072 mmol) were dissolved in 2 mL of anhydrous DCM. The reaction mixture was stirred for 46 h before being quenched. The crude product was partially purified by column chromatography (Hexane:EtOAc 6:1), before being dissolved in a mixture of 1 M solution of TBAF in THF (1 mL) and THF (2 mL). The resulting reaction mixture was left to stir for 2 h 15 min at RT, until the reaction was judged to be complete by TLC. Solvent was removed under reduced pressure and the product purified by column chromatography (Hexane:EtOAc 6:1 → 11:9) to afford the title compound **213** as a yellow oil (0.091 g, 64 %), with spectroscopic details in accordance with the literature;[108] **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 7.41 – 7.26 (25 H, m, H_{arom}), 5.57 (1 H, app d, *J* 3.9, H-1'), 5.05 (1 H, d, *J* 11.0, PhC*H*H), 4.89 (1 H, d, *J* 11.9, PhC*H*H), 4.78 (1 H, d, *J* 12.0, PhC*H*H), 4.71 (1 H, d, *J* 11.0, PhCH*H*), 4.65 (1 H, d, *J* 12.1, PhCH*H*), 4.61 (1 H, d, *J* 3.5, H-1), 4.58 (1 H, d, *J* 11.9, PhCH*H*), 4.58 (2 H, s, PhCH_2), 4.54 (1 H, d, *J* 12.2, PhC*H*H), 4.44 (1 H, d, *J* 12.2, PhCH*H*), 3.95 (1 H, t, *J* 9.2, H-3), 3.89 (1 H, t, *J* 9.4, H-4), 3.89 – 3.82 (3 H, m, H-3', H-5', H-6a), 3.78 (1 H, bs, H-4'), 3.68 (1 H, ddd, *J* 12.5, 6.4, 2.1, H-6b), 3.63 – 3.56 (2 H, m, H-2, H-6a'), 3.55 (1 H, dt, *J* 9.7, 2.4, H-5), 3.39 (3 H, s, OCH_3), 3.33 (1 H, t, *J* 6.6, OH), 3.26 (1 H, dd, *J* 9.8, 3.7, H-6b'), 2.15 (1 H, td, *J* 12.5, 4.2, H-2ax'), 1.88 (1 H, ddt, *J* 12.8, 4.6, 1.4, H-2eq'); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) 138.59, 138.49, 138.42, 138.08, 137.33 (5 4° C_{arom}), 128.50, 128.41, 128.31, 128.26, 128.24, 128.00, 127.95, 127.75, 127.72, 127.68, 127.65, 127.33 (25 C_{arom}), 99.76 (C-

1'), 98.20 (C-1), 82.29 (C-3), 80.07 (C-2), 75.62 (Ph*C*H_2), 74.70 (C-3'), 74.46 (C-5'), 73.95 (Ph*C*H_2), 73.65 (Ph*C*H_2), 73.37 (Ph*C*H_2), 72.96 (C-4'), 71.59 (C-4), 70.71, 70.66, 70.60 (Ph*C*H_2, C-5, C-6'), 60.53 (C-6), 55.23 (OCH_3), 31.32 (C-2'); **m/z** (ESI-MS+) $C_{48}H_{54}O_{10}Na^+$ ([M + Na]$^+$) calculated 813.4; found 813.4.

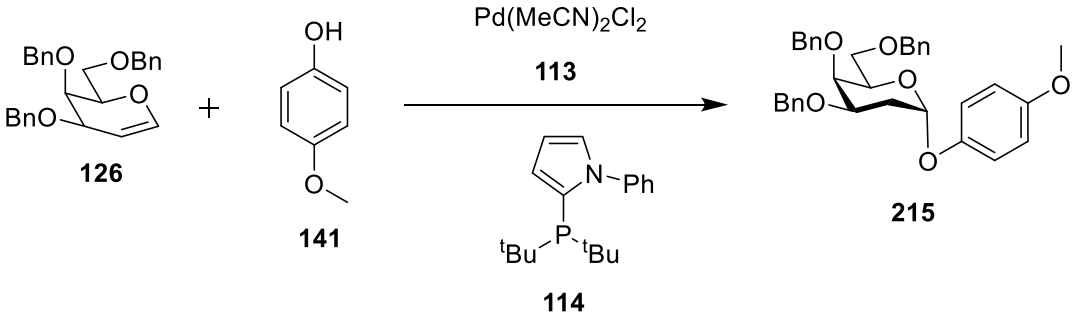
Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-benzyl-α-D-lyxo-hexapyranosyl)-β-D-galactopyranoside 214



Following the general procedure for palladium catalysed glycosylation reactions, glycosyl donor tri-*O*-benzyl-D-galactal **126** (0.100 g, 0.240 mmol), glycosyl acceptor methyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside **121** (0.084 g, 0.180 mmol), metal catalyst bis(acetonitrile)dichloropalladium (II) **113** (0.019 g, 0.072 mmol) and ligand 2-(di-*tert*-butylphosphanyl)-1-phenyl-1H-pyrrole **114** (0.021 g, 0.072 mmol) were dissolved in 2 mL of anhydrous DCM. The reaction mixture was stirred for 45 h before being quenched. Following purification by column chromatography (Hexane:EtOAc 8:2) the title compound **214** was obtained as a yellow oil (0.112 g, 70 %, α:β = 3.5:1), with spectroscopic details in accordance with the literature.[108] Note that only characteristic, non-overlapping, distinguishable NMR spectroscopy signals are given for the minor β anomer; **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) **α anomer:** 7.42 – 7.16 (30 H, m, H_{arom}), 5.06 (1 H, app d, *J* 3.4, H-1'), 4.93 (1 H, d, *J* 11.5, PhC*H*H), 4.89 (1 H, d, *J* 11.5, PhC*H*H), 4.87 (1 H, d, *J* 11.5, PhC*H*H), 4.80 (1 H, d, *J* 11.0, PhCH*H*), 4.65 (1 H, d, *J* 11.4, PhC*H*H), 4.61 (1 H, d, *J* 11.2, PhCH*H*), 4.57 (1 H, d, *J* 11.4, PhCH*H*), 4.55 (1 H, d, *J* 12.9, PhCH*H*), 4.51 (2 H, s, Ph*CH*$_2$), 4.41 (1 H, ddd, *J* 9.1, 4.8, 1.3, H-5'), 4.24 (1 H, d, *J* 7.7, H-1), 4.15 (2 H, s, Ph*CH*$_2$), 4.13 (1 H, d, *J* 3.1, H-4), 4.03 (1 H, bs, H-4'), 4.00 (1 H, ddd, *J* 12.0, 4.5, 2.4, H-3'), 3.66 – 3.58 (4 H, m, H-6a', H-2, H-6a, H-6b), 3.57 (3 H, s, OCH_3), 3.57 – 3.52 (1 H, m, H-5), 3.39 (1 H, dd, *J* 10.0, 3.1, H-3), 3.28 (1 H, dd, *J* 8.4, 4.8, H-6b'), 2.21 (1 H, td, *J* 12.2, 3.7, H-2ax'), 2.03 (1 H, ddt, *J* 12.4, 4.5, 1.4, H-2eq'); **β anomer:** 5.27 (1 H, app d, *J* 3.4,

H-1'), 2.26 (1 H, td, *J* 12.4, 3.7, H-2ax'), 1.87 (1 H, dd, *J* 12.6, 4.5, H-2eq'); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) **α anomer:** 139.31, 138.88, 138.81, 138.66, 138.26, 137.44 (6 4° C_{arom}), 128.67, 128.50, 128.37, 128.36, 128.34, 128.24, 128.21, 128.17, 128.15, 127.97, 127.71, 127.59 (24 C_{arom}), 105.28 (C-1), 99.80 (C-1'), 80.24 (C-3), 78.90 (C-2), 75.14 (Ph*C*H_2), 74.63, 74.55 (Ph*C*H_2, C-3'), 73.87 (Ph*C*H_2), 73.29 (Ph*C*H_2), 73.17, 73.15 (C-4, C-5), 72.89 (C-4'), 72.11 (Ph*C*H_2), 70.59 (Ph*C*H_2), 69.75 (C-5'), 68.52 (C-6'), 68.02 (C-6), 57.57 (OCH_3), 31.65 (C-2'); **β anomer:** 93.52 (C-1'); **m/z** (ESI-MS+) $C_{55}H_{60}O_{10}Na^+$ ([M + Na]$^+$) calculated 903.41; found 903.41; $C_{47}H_{50}O_8Na^+$ ([M + Na - BnO - MeO]$^+$) calculated 765.34; found 765.35.

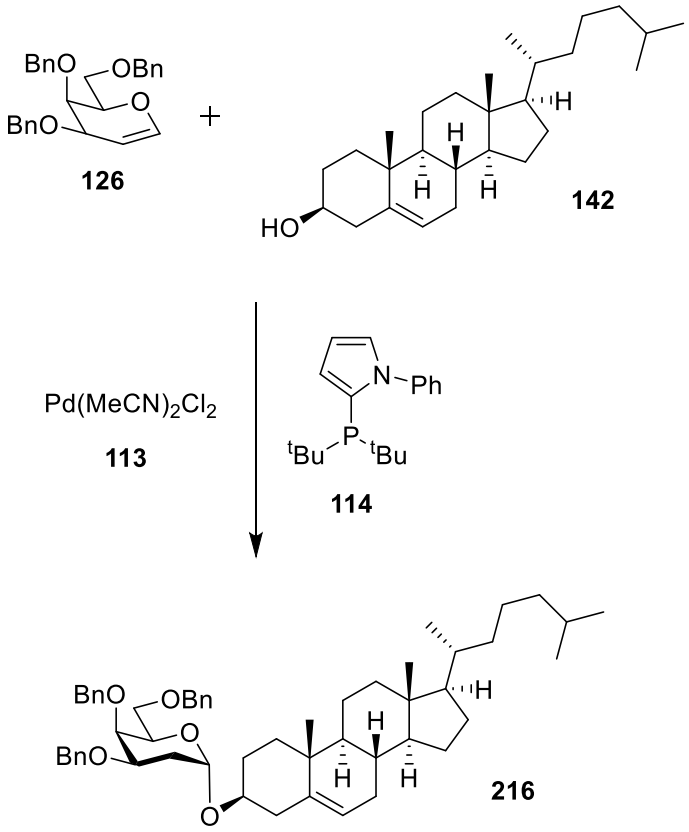
(*p*-Methoxy) phenyl 3,4,6-tri-*O*-benzyl-α-D-lyxo-hexapyranoside 215



Following the general procedure for palladium catalysed glycosylation reactions, glycosyl donor tri-*O*-benzyl-D-galactal **126** (0.100 g, 0.240 mmol), glycosyl acceptor (*p*-methoxy) phenol **141** (0.022 g, 0.180 mmol), metal catalyst bis(acetonitrile)dichloropalladium (II) **113** (0.019 g, 0.072 mmol) and ligand 2-(di-*tert*-butylphosphanyl)-1-phenyl-1H-pyrrole **114** (0.021 g, 0.072 mmol) were dissolved in 2 mL of anhydrous DCM. The reaction mixture was stirred for 24 h before being quenched. Following purification by column chromatography (Hexane:EtOAc 9:1) the title compound **215** was obtained as a yellow oil (0.062 g, 64 %, α:β = 2:1) with spectroscopic details in accordance with the literature.[184] Note that only characteristic, non-overlapping, distinguishable NMR spectroscopy signals are given for the minor β anomer; **^{1}H NMR α anomer:** δ_H (500 MHz, Chloroform-*d*) 7.42 – 7.20 (15 H, m, H_{arom}), 6.99 (2 H, m, $C_6H_4OCH_3$), 6.79 (2 H, m, $C_6H_4OCH_3$), 5.59 (1 H, app d, *J* 3.3, H-1), 4.97 (1 H, d, *J* 11.5, PhC*H*H), 4.68 (2 H, s, PhCH_2), 4.65 (1 H, d, *J* 11.6, PhCH*H*), 4.43 (1 H, d, *J* 11.6, PhC*H*H), 4.37 (1 H, d, *J* 11.6, PhCH*H*), 4.13 (1 H, ddd, *J* 12.1, 4.6, 2.5, H-3), 4.09 (1 H, t, *J* 6.5, H-5), 4.01 (1 H, d, *J* 2.3, H-4), 3.76 (3 H, s, OCH_3), 3.69 – 3.53 (2 H, m, H-6a, H-6b), 2.38 (1 H, td, *J* 12.4, 3.7, H-2ax), 2.21 (1 H, ddt, *J* 12.8, 4.7, 1.4, H-2eq); **β anomer:** δ_H (500 MHz, Chloroform-*d*)

5.09 (1 H, d, *J* 3.5, H-1), 2.25 (1 H, td, *J* 12.4, 3.8, H-2a), 2.05 (1 H, dd, *J* 12.6, 4.8, H-2b); **^{13}C NMR α anomer:** δ $_C$ (126 MHz, Chloroform-*d*) 154.86 (4˚ $C_{arom}OCH_3$), 151.06 (4˚ $C_{arom}O$(C-1)), 138.96, 138.61, 138.21 (3 4˚ C_{arom}(Bn)), 128.58, 128.45 - 127.48 (15 C_{arom}), 118.16 (2 $C_6H_4OCH_3$), 114.63 (2 $C_6H_4OCH_3$), 97.57 (C-1), 74.75 (C-3), 74.54 (PhCH_2), 73.48 (PhCH_2), 73.09 (C-4), 70.71, 70.70 (PhCH_2, C-5), 69.51 (C-6), 55.76 (OCH_3), 31.47 (C-2); **β anomer:** δ $_C$ (126 MHz, Chloroform-*d*) 97.25 (C-1); **m/z** (ESI-MS+) $C_{34}H_{36}O_6Na^+$ ([M + Na]$^+$) calculated 563.2; found 563.2.

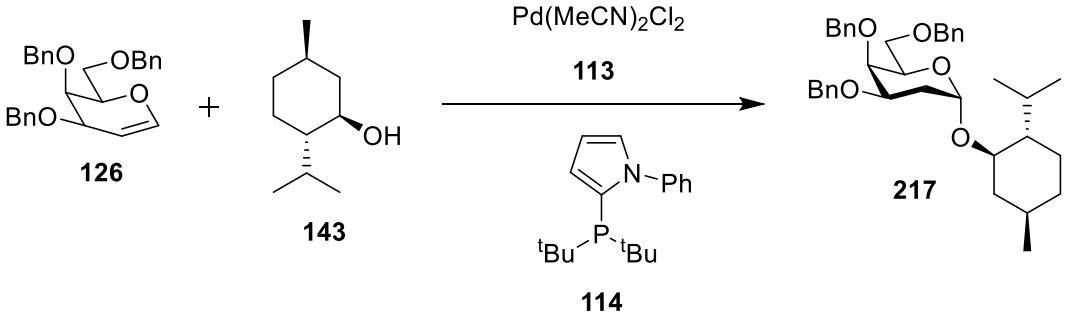
Cholesteryl 3,4,6-tri-*O*-benzyl-α-D-lyxo-hexapyranoside 216



Following the general procedure for palladium catalysed glycosylation reactions, glycosyl donor tri-*O*-benzyl-D-galactal **126** (0.100 g, 0.240 mmol), glycosyl acceptor cholesterol **142** (0.035 g, 0.180 mmol), metal catalyst bis(acetonitrile)dichloropalladium (II) **113** (0.019 g, 0.072 mmol) and ligand 2-(di-*tert*-butylphosphanyl)-1-phenyl-1H-pyrrole **114** (0.021 g, 0.072 mmol) were dissolved in 2 mL of anhydrous DCM. The reaction mixture was stirred for 44 h before being quenched. Following purification by column chromatography (Hexane:EtOAc 10:1) the title compound **216** was obtained as a yellow oil (0.060 g, 42 %,

α:β = 6:1), with spectroscopic details in accordance with the literature;[185] **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 7.38 – 7.23 (15 H, m, H_{arom}), 5.27 (1 H, app d, *J* 4.8, C=CH (cholesteryl)), 5.15 (1 H, app d, *J* 3.6, H-1), 4.94 (1 H, d, *J* 11.6, PhC*H*H), 4.65 – 4.60 (3 H, m, PhCH_2), 4.51 (1 H, d, *J* 11.7, PhC*H*H), 4.44 (1 H, d, *J* 11.8, PhCH*H*), 4.01 (1 H, t, *J* 6.5, H-5), 3.97 (1 H, ddd, *J* 11.9, 4.5, 2.5, H-3), 3.95 (1 H, app bs, H-4), 3.60 (2 H, qd, *J* 9.5, 6.4, H-6a, H-6b), 3.50 – 3.42 (1 H, m, OCH (cholesteryl)), 2.29 (2 H, d, *J* 7.6, OCHCH_2C=C (cholesteryl)), 2.24 (1 H, td, *J* 12.2, 3.8, H-2ax), 2.01 (1 H, dt, *J* 12.6, 3.6, cholesteryl), 1.99 – 1.91 (2 H, m, H-2eq, CH (cholesteryl)), 1.91 – 1.78 (3 H, m, cholesteryl), 1.64 – 1.30 (10 H, m, cholesteryl), 1.30 – 1.24 (2 H, m, cholesteryl), 1.21 – 0.95 (12 H, m, cholesteryl), 0.92 (3 H, d, *J* 6.5, CH_3 (cholesteryl)), 0.88 (6 H, dd, *J* 6.6, 2.2, CH(CH_3)$_2$ (cholesteryl)), 0.68 (3 H, s, CH_3 (cholesteryl)); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) 141.05 (OCHCH$_2$*C*=C (cholesteryl)), 139.13, 138.79, 138.31 (3 4° C_{arom}), 128.51, 128.49, 128.32, 127.88, 127.74, 127.60, 127.58, 127.44 (15 C_{arom}), 121.76 (OCHCH$_2$C=*C* (cholesteryl)), 95.84 (C-1), 76.27 (OCH (cholesteryl), 75.22 (C-3), 74.41 (Ph*C*H$_2$), 73.57 (Ph*C*H$_2$), 73.31 (C-4), 70.63 (Ph*C*H$_2$), 69.99 (C-5), 69.80 (C-6), 56.93, 56.30, 50.26, 42.48, 40.18, 39.93, 39.68, 37.26, 36.86, 36.35, 35.94, 32.09, 32.05 (13 C (cholesteryl)), 31.84 (C-2), 28.39, 28.17, 27.97, 24.46, 23.98 (5 C (cholesteryl)), 22.98, 22.72 (2 CH(*C*H$_3$)$_2$ (cholesteryl)), 21.20, 19.53 (2 C (cholesteryl)), 18.87, 12.01 (2 CH_3 (cholesteryl)); **m/z** (ESI-MS+) $C_{54}H_{74}O_5Na^+$ ([M + Na]$^+$) calculated 825.5; found 825.6.

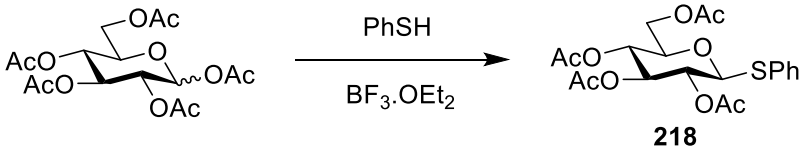
Menthyl 3,4,6-tri-*O*-benzyl-α-D-lyxo-hexapyranoside 217



Following the general procedure for palladium catalysed glycosylation reactions, glycosyl donor tri-*O*-benzyl-D-galactal **126** (0.100 g, 0.240 mmol), glycosyl acceptor (-)-menthol **143** (0.028 g, 0.180 mmol), metal catalyst bis(acetonitrile)dichloropalladium (II) **113** (0.019 g, 0.072 mmol) and ligand 2-(di-*tert*-butylphosphanyl)-1-phenyl-1H-pyrrole **114**

(0.021 g, 0.072 mmol) were dissolved in 2 mL of anhydrous DCM. The reaction mixture was stirred for 42 h before being quenched. Following purification by column chromatography (Hexane:EtOAc 13:1) the title compound **217** was obtained as a colourless solid (0.031 g, 30 %, α:β = 6:1). Note that only characteristic, non-overlapping, distinguishable NMR spectroscopy signals are given for the minor β anomer; **^{1}H NMR α anomer:** δ_H (500 MHz, Chloroform-*d*) 7.40 – 7.21 (15 H, m, H_{arom}), 5.03 (1 H, app d, *J* 3.5, H-1), 4.94 (1 H, d, *J* 11.7, PhC*H*H), 4.65 – 4.61 (3 H, m, PhCH_2), 4.51 (1 H, d, *J* 11.9, PhC*H*H), 4.44 (1 H, d, *J* 12.1, PhCH*H*), 4.07 (1 H, t, *J* 6.5, H-5), 3.96 – 3.91 (2 H, m, H-3, H-4), 3.58 (2 H, dd, *J* 6.5, 3.0, H-6a, H-6b), 3.30 (1 H, td, *J* 10.6, 4.4, OCH (menthyl)), 2.24 – 2.16 (1 H, m, H-2ax), 2.13 – 2.07 (1 H, m, OCHC*H*H (menthyl)), 2.04 (1 H, td, *J* 7.1, 2.5, OCHCHC*H*$(CH_3)_2$ (menthyl)), 2.00 (1 H, ddt, *J* 12.5, 4.6, 1.5, H-2eq), 1.66 – 1.57 (2 H, m, $OCHCH_2CH(CH_3)$C*H*H (menthyl), OCHCH(^{i}Pr)C*H*H (menthyl)), 1.42 – 1.29 (1 H, m, $OCHCH_2$C*H*(CH_3) (menthyl)), 1.18 (1 H, ddt, *J* 13.3, 10.3, 3.0, OCHC*H*(^{i}Pr) (menthyl)), 0.98 – 0.86 (5 H, m, OCHCH*H* (menthyl), $OCHCH_2CH(CH_3)$CH*H* (menthyl), CH(CH_3)(CH_3) (menthyl)), 0.82 (3 H, d, *J* 6.6, $OCHCH_2CH$(CH_3) (menthyl)), 0.81 – 0.78 (1 H, m, OCHCH(^{i}Pr)CH*H* (menthyl)), 0.76 (3 H, d, *J* 7.0, CH(CH_3)(CH_3) (menthyl)); **β anomer:** δ_H (500 MHz, Chloroform-*d*) 4.49 – 4.47 (1 H, m, H-1); **^{13}C NMR α anomer:** δ_C (126 MHz, Chloroform-*d*) 139.17, 138.81, 138.40 (3 4° C_{arom}), 128.51, 128.48, 128.32, 128.30, 127.75, 127.72, 127.61, 127.57, 127.45 (15 C_{arom}), 99.95 (C-1), 80.16 (OCH (menthyl)), 75.13 (C-3), 74.38 (Ph*C*H_2), 73.55 (Ph*C*H_2), 73.40 (C-4), 70.54 (Ph*C*H_2), 70.06 (C-5), 69.89 (C-6), 49.06 (OCH*C*H(^{i}Pr) (menthyl)), 43.07 (OCH*C*H_2 (menthyl)), 34.54 (OCHCH(^{i}Pr)*C*H_2 (menthyl)), 31.86, 31.83 (C-2, $OCHCH_2$*C*H(CH_3) (menthyl)), 25.90 (OCHCH*C*H$(CH_3)_2$ (menthyl)), 23.45 ($OCHCH_2CH(CH_3)$*C*H_2 (menthyl)), 22.42 ($OCHCH_2CH$(*C*H_3) (menthyl)), 21.33 (CH(*C*H_3)(CH_3) (menthyl)), 16.50 (CH(CH_3)(*C*H_3) (menthyl)); **β anomer:** δ_C (126 MHz, Chloroform-*d*) 97.59 (C-1); **m/z** (ESI-HRMS) $C_{37}H_{48}O_5Na^+$ ([M + Na]$^+$) calculated 595.3394; found 595.3386; **IR** ν_{max}/cm^{-1} 3028, 2945, 2919, 2867, 1496, 1454, 1357, 1203, 1166, 1093, 1061, 1027, 734, 697.

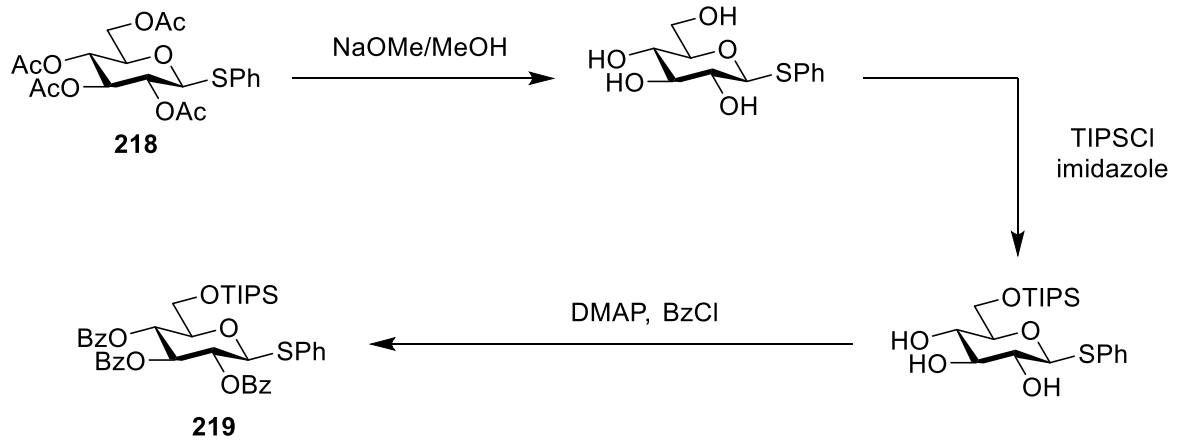
Phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-thioglucopyranoside 218



218

1,2,3,4,6-Penta-*O*-acetyl-β-D-glucopyranoside (10.00 g, 25.62 mmol) was dried under vacuum for 1 h before being dissolved in anhydrous dichloromethane (50 mL) and cooled to 0 ℃ under a nitrogen atmosphere. To this solution, thiophenol (5.3 mL, 51.24 mmol) was added, followed by boron trifluoride diethyl etherate (9.5 mL, 76.86 mmol) dropwise. The resulting solution was stirred at RT for 18 h. The mixture was then cautiously quenched using $NaHCO_{3\ (sat.\ aq.)}$. The product was extracted from the reaction mixture using further portions of DCM (2 x 50 mL) and the combined DCM phase was washed with brine (3 x 50 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. The crude residue was purified by recrystallisation from diethyl ether to provide the title compound **218** (8.39 g, 74 %) as a white solid with spectroscopic details in accordance with the literature;[108] **^{1}H NMR** δ_H (400 MHz, Chloroform-*d*) 7.52 – 7.46 (2 H, m, H_{arom}), 7.34 – 7.28 (3 H, m, H_{arom}), 5.22 (1 H, t, *J* 9.4, H-3), 5.04 (1 H, t, *J* 9.8, H-4), 4.97 (1 H, dd, *J* 10.1, 9.2, H-2), 4.70 (1 H, d, *J* 10.1, H-1), 4.21 (1 H, d, *J* 5.0, H-6a), 4.17 (1 H, dd, *J* 12.3, 2.7, H-6b), 3.72 (1 H, ddd, *J* 10.1, 5.0, 2.7, H-5), 2.08 (3 H, s, CH_3), 2.08 (3 H, s, CH_3), 2.01 (3 H, s, CH_3), 1.99 (3 H, s, CH_3); **^{13}C NMR** δ_C (101 MHz, Chloroform-*d*) 170.72, 170.32, 169.53, 169.39 (4 C=O), 133.25 (4˚ C_{arom}), 131.76, 129.07, 128.55 (C_{arom}), 85.86 (C-1), 75.92 (C-5), 74.09 (C-3), 70.06 (C-2), 68.33 (C-4), 62.27 (C-6), 20.88, 20.86, 20.72, 20.71 (4 CH_3); **m/z** (ESI-MS+) $C_{20}H_{24}O_9Na^+$ ([M + Na]$^+$) calculated 463.10; found 463.10.

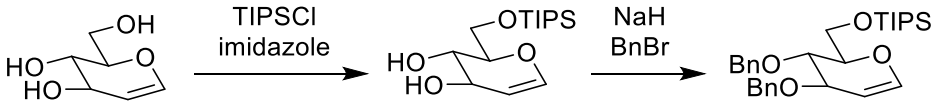
Phenyl 2,3,4-tri-*O*-benzoyl-6-*O*-triisopropylsilyl-β-D-thioglucopyranoside 219



Phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-thioglucopyranoside **218** (6.48 g, 14.71 mmol) was dried under vacuum for 1 h before being dissolved in methanol (50 mL) and cooled to 0 ℃. Sodium methoxide (0.34 mL, 1.47 mmol, 25 % wt in MeOH) was added dropwise and the

resulting solution was stirred at 0 °C for 40 min after which time TLC (DCM:MeOH 9:1) showed the reaction to be complete. The solution was then brought to pH 7, as monitored by universal indicator paper, using 1 M $HCl_{(aq.)}$. Solvent was removed from the resulting neutralised solution under reduced pressure to give a crude mass of 4.29 g of impure phenyl-β-D-thioglucopyranoside containing NaCl salts. A 2.14 g portion of this impure phenyl-β-D-thioglucopyranoside (approx. 7.86 mmol) was dried for 1 h under vacuum before being dissolved in anhydrous pyridine (50 mL) under a nitrogen atmosphere. To this solution, imidazole (1.07 g, 15.72 mmol) was added and the solution was cooled to 0 °C. Triisopropylsilyl chloride (2.02 mL, 9.43 mmol) was added dropwise and the reaction was stirred at RT for 18 h. After this time, TLC (DCM:MeOH 95:5) showed the silylation reaction to be complete. Benzoyl chloride (7.30 mL, 62.88 mmol) and DMAP (0.097 g, 0.79 mmol) were then added, and the solution was stirred at RT for 26 h under a nitrogen atmosphere. After this time, TLC (Hexane:EtOAc 9:1) showed the benzoylation reaction to be complete. Methanol (15 mL) was cautiously added to quench excess benzoyl chloride, then the reaction mixture was diluted with DCM (150 mL). The resulting organic solution was washed with 1 M $HCl_{(aq.)}$ (2 x 100 mL), $NaHCO_{3\ (sat.\ aq.)}$ (100 mL) and water (100 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (Hexane:EtOAc 93:7 → 85:15) to give the title compound **219** (4.87 g, 90 % over 3 steps), with spectroscopic details in accordance with the literature;[108] **^{1}H NMR** δ_H (400 MHz, Chloroform-*d*) 7.96 – 7.91 (2 H, m, H_{arom}), 7.91 – 7.86 (2 H, m, H_{arom}), 7.80 – 7.75 (2 H, m, H_{arom}), 7.54 – 7.41 (4 H, m, H_{arom}), 7.38 – 7.17 (10 H, m, H_{arom}), 5.87 (1 H, t, *J* 9.5, H-3), 5.54 (1 H, t, *J* 9.2, H-4), 5.46 (1 H, t, *J* 9.7, H-2), 5.04 (1 H, d, *J* 10.0, H-1), 3.97 – 3.87 (3 H, m, H-5, H-6a, H-6b), 1.10 – 0.95 (21 H, m, TIPS); **^{13}C NMR** δ_C (101 MHz, Chloroform-*d*) 165.95, 165.17, 165.16 (3 C=O), 133.33, 133.19, 132.70, 132.61 (4 4° C_{arom}), 129.93, 129.83, 129.79, 129.40, 129.24, 129.01, 128.98, 128.44, 128.42, 128.31, 128.09 (C_{arom}), 86.46 (C-1), 79.99 (C-5), 74.72 (C-3), 70.78 (C-2), 69.34 (C-4), 63.07 (C-6), 18.01 ($CH(\mathit{C}H_3)_2$), 11.97 ($\mathit{C}H(CH_3)_2$); **m/z** (ESI-MS+) $C_{42}H_{48}O_8SSiNa^+$ ($[M + Na]^+$) calculated 763.3; found 763.3; $C_{22}H_{35}O_4Si^+$ ($[M - SPh - 2\ OBz + 2\ H]^+$) calculated 391.2; found 391.3.

3,4-Di-*O*-benzyl-6-*O*-(triisopropylsilyl)-D-glucal 220

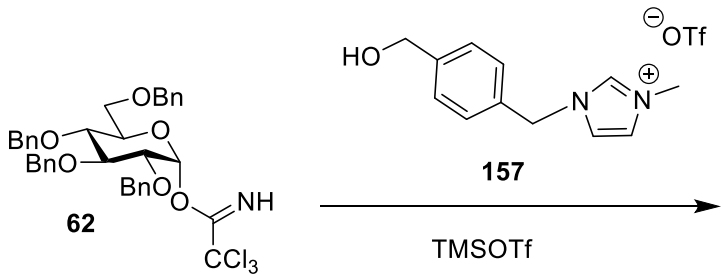


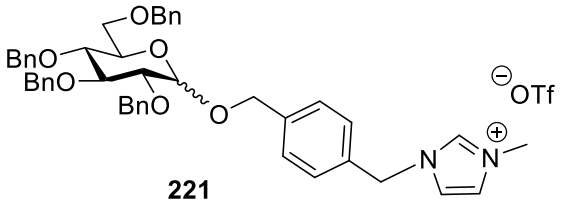
220

D-Glucal (10.00 g, 68.43 mmol) was dried under vacuum for 1 h in a flame-dried flask before being dissolved in anhydrous DMF (160 mL) under a nitrogen atmosphere. Imidazole (9.32 g, 136.85 mmol) was added and the resulting solution was cooled to 0 ˚C. TIPSCl (19.06 mL, 88.95 mmol) was added dropwise and the reaction was left to stir under a nitrogen atmosphere for 18 h at RT. The solvent was removed under reduced pressure and the residue was dissolved in DCM (250 mL), then washed with water (250 mL). The aqueous phase was washed with DCM (2 x 100 mL) and DCM fractions were combined. The DCM phase was washed with brine (200 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. The crude residue was partially purified by column chromatography (Hexane:EtOAc 85:15 → 6:4), to give impure intermediate 6-*O*-(triisopropylsilyl)-D-glucal. This intermediate was dried under vacuum for 1 h in a flame-dried flask before being dissolved in anhydrous DMF (160 mL) under a nitrogen atmosphere and cooled to 0 ˚C. Sodium hydride (60 % in mineral oil, 13.68 g, 342.15 mmol) was added and the mixture stirred for 60 min at RT, after which time the mixture was again cooled to 0 ℃. Benzyl bromide (24.4 mL, 205.3 mmol) was added dropwise and the reaction mixture stirred for 18 h at RT, after which time methanol (15 mL) was added cautiously. The solvent was removed under reduced pressure and the residue was dissolved in DCM (250 mL), then washed with water (2 x 150 mL). The combined aqueous phase was washed with DCM (100 mL) and the DCM fractions were combined. The DCM phase was washed with brine (200 mL), dried using magnesium sulfate, filtered and the solvent was removed under reduced pressure. Following purification by normal phase HPLC (Hexane:EtOAc), the title compound **220** was obtained as an oil (20.47 g, 61 % over 2 steps) with spectroscopic details in accordance with the literature;[186] **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 7.43 – 7.34 (10 H, m, H_{arom}), 6.43 (1 H, dd, *J* 6.2, 1.4, H-1), 4.91 (1 H, d, *J* 11.3, PhC*H*H), 4.88 (1 H, dd, *J* 6.1, 2.7, H-2), 4.81 (1 H, d, *J* 11.3, PhCH*H*), 4.68 (1 H, d, *J* 11.7, PhC*H*H), 4.62 (1 H, d, *J* 11.9, PhCH*H*), 4.25 (1 H, ddt, *J* 5.5, 2.7, 1.4, H-3), 4.09 (1 H, dd, *J* 11.2, 3.6, H-6a), 4.04 (1 H, dd, *J* 11.2, 2.5, H-6b), 4.01 – 3.94 (2 H, m,

H-4, H-5), 1.15 – 1.08 (21 H, m, TIPS); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) 144.89 (C-1), 138.60, 138.57 (2 4° C_{arom}), 128.51, 128.50, 128.49, 128.02, 127.89, 127.88, 127.79, 127.74, 127.71 (C_{arom}), 99.71 (C-2), 78.25 (C-4 or C-5), 75.81 (C-3), 74.17 (C-4 or C-5), 73.93 (Ph*C*H_2), 70.74 (Ph*C*H_2), 62.08 (C-6), 18.12, 18.08, 12.12 (TIPS); **m/z** (ESI-MS+) $C_{29}H_{42}O_4SiNa^+$ ([M + Na]$^+$) calculated 505.27; found 505.27; $C_{22}H_{35}O_3Si^+$ ([M - OBn]$^+$) calculated 375.24; found 375.23.

4-(1-Methyl-3-methyleneimidazolium)benzyl 2,3,4,6-tetra-*O*-benzyl-D-glucopyranoside trifluoromethanesulfonate 221

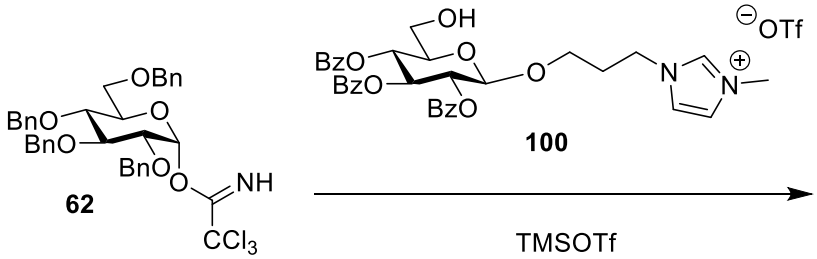


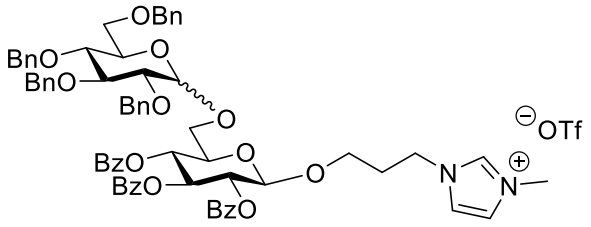


Following the general procedure for I-Tag glycosylations in flow using glycosyl acceptor 1-(4-(hydroxymethyl)benzyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate **157** (0.0705 g, 0.200 mmol, 1 eq) and glycosyl donor 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl trichloroacetimidate **62** (0.4110 g, 0.600 mmol, 3.0 eq). 1.0 mL of anhydrous acetonitrile was added to the donor/acceptor vial, resulting in a solution of volume 1.3 mL and therefore approximately 0.154 M in acceptor and 0.462 M in donor. To the other vial, 1.5 mL of anhydrous acetonitrile was added, followed by trimethylsilyl trifluoromethanesulfonate (32.5 μL, 0.180 mmol) to make a 0.12 M solution. Reaction solution was collected for a total of 17 min. The crude product was washed with water (2 x 2.5 mL), then the water was extracted with DCM (3 x 5 mL). The dried residue was washed with hexane (3 x 5 mL) and hexane:Et_2O 1:1 (6 x 5 mL) then dried under reduced pressure to yield the title compound **221** as an oil (0.1350 g, 90 %, α:β = 1:2); **^{1}H NMR** δ_H (500 MHz, Acetonitrile-d_3) 8.56 (2 H, bs, NCHN α and β), 7.48 – 7.20 (m, H_{arom}), 5.03 (1 H, d, *J* 3.5, H-1 α),

4.92 – 4.46 (m), 3.81 (3 H, s, NCH_3 β), 3.80 (3 H, s, NCH_3 α), 3.86 – 3.46 (m), 3.40 (1 H, dd, *J* 8.5, 7.9, H-2 β); **^{13}C NMR** δ_C (126 MHz, Acetonitrile-d_3) 140.04, 139.99, 139.87, 139.71, 139.58, 139.53, 139.49, 139.45 (4˚ C_{arom}), 137.08 (NCHN α and β), 134.23, 134.19 (4˚ C_{arom}), 129.69, 129.60, 129.54, 129.50, 129.27, 129.26, 129.21, 129.19, 129.16, 128.90, 128.84, 128.83, 128.79, 128.78, 128.75, 128.74, 128.72, 128.59, 128.54, 128.50, 128.50, 128.46, 128.39, 128.36, 128.25 (C_{arom}), 124.96 (N*C*HCHN), 124.93 (N*C*HCHN), 123.14 (N*C*HCHN), 103.35 (C-1 β), 96.96 (C-1 α), 85.27, 83.08 (C-2 β), 82.56, 81.09, 78.83, 78.77, 75.95, 75.87, 75.52, 75.44, 75.40, 75.16, 73.79, 73.78, 73.15, 71.53, 71.03, 69.88, 69.82, 69.45, 36.89 (NCH_3), 36.88 (NCH_3); **^{19}F NMR** δ_F (470 MHz, Acetonitrile-d_3) -79.18 ($^-$OTf); **m/z** (ESI-HRMS) $C_{46}H_{49}N_2O_6^+$ ([M – OTf]$^+$) calculated: 725.3585; found 725.3583; **IR** ν_{max}/cm^{-1} 3148w, 3064w, 3031w, 2867w, 1574w, 1497, 1454, 1361, 1259s, 1225, 1160, 1070, 1030s, 913w, 826w, 741, 699, 638, 623.

3-(3-Methylimidazolium)-propyl 2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-β-D-glucopyranoside trifluoromethanesulfonate 222



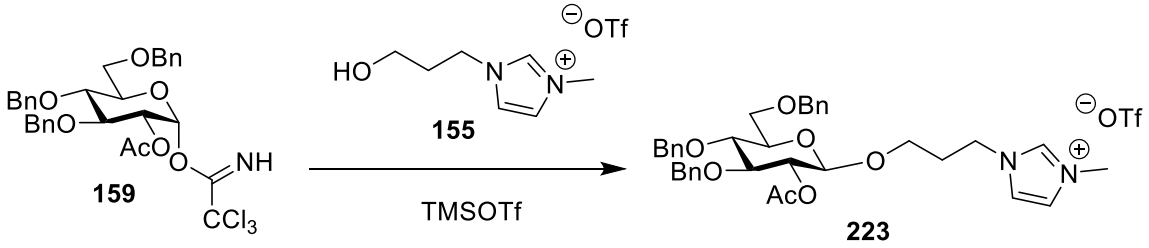


222

Following the general procedure for I-Tag glycosylations in flow using glycosyl acceptor 3-(3-methylimidazolium)-1-propyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside trifluoromethanesulfonate **100** (0.1529 g, 0.200 mmol, 1 eq) and glycosyl donor 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl trichloroacetimidate **62** (0.2740 g, 0.400 mmol, 2.0 eq). 1.0 mL

of anhydrous acetonitrile was added to the donor/acceptor vial, resulting in a solution of volume 1.20 mL and therefore approximately 0.167 M in acceptor and 0.333 M in donor. To the other vial, 1.5 mL of anhydrous acetonitrile was added, followed by trimethylsilyl trifluoromethanesulfonate (16.3 µL, 0.090 mmol) to make a 0.06 M solution. Reaction solution was collected for a total of 15 min. The crude product was washed with water (3 mL), then the water was extracted with DCM (2 x 15 mL). The dried residue was washed with Hexane:Et_2O 1:1 (3 x 5 mL) then dried under reduced pressure, before being passed through a silica plug with DCM:MeOH 93:7, yielding the title compound **222** as a solid (0.1825 g, 86 %, α:β = 1:2.5); **^{1}H NMR** δ_H (500 MHz, Acetonitrile-d_3) 8.27 (1 H, s, NCHN β), 8.13 (1 H, s, NCHN α), 7.93 – 7.86 (m, H_{arom}), 7.75 (m, H_{arom}), 7.62 – 7.49 (m, H_{arom}), 7.47 – 7.19 (m, H_{arom}), 7.19 – 7.17 (1 H, m, NC*H*CHN β), 7.10 (1 H, t, *J* 1.8, NC*H*CHN α), 5.86 (m), 5.62 – 5.52 (m), 5.33 (1 H, dd, *J* 9.8, 8.0, H-2 β), 5.23 (1 H, dd, *J* 9.7, 8.0, H-2 α), 4.99 – 4.94 (m, H-1' α), 4.92 – 4.87 (m, H-1 β, H-1 α), 4.86 – 4.73 (m), 4.72 – 4.65 (m), 4.54 (1 H, d, *J* 10.9), 4.51 (1 H, d, *J* 7.8, H-1' β), 4.50 – 4.44 (m), 4.39 (1 H, d, *J* 11.9), 4.34 (1 H, d, *J* 11.9), 4.22 – 4.15 (m), 4.09 (1 H, dd, *J* 11.5, 2.5), 4.06 – 3.95 (m), 3.95 – 3.81 (m), 3.79 – 3.74 (m), 3.76 (3 H, s, NCH_3 β), 3.75 (3 H, s, NCH_3 α), 3.72 – 3.43 (m), 3.39 (1 H, dd, *J* 10.9, 2.0), 3.32 (1 H, dd, *J* 9.0, 7.8, H-2' β), 1.92 – 1.86 (m, $CH_2CH_2CH_2$); **^{13}C NMR** δ_C (126 MHz, Acetonitrile-d_3) 166.36, 166.35, 166.11, 166.01, 166.00 (C=O), 139.85, 139.59, 139.54, 139.46, 139.40, 139.32 (C_{arom}), 136.95 (NCHN β), 136.92 (NCHN α), 134.67, 134.66, 134.56, 130.49, 130.44, 130.34, 130.15, 130.14, 130.08, 129.98, 129.95, 129.87, 129.86, 129.68, 129.59, 129.57, 129.53, 129.30, 129.27, 129.24, 129.22, 129.18, 129.00, 128.87, 128.77, 128.75, 128.68, 128.57, 128.52, 128.51, 128.49, 128.43 (C_{arom}), 124.44 (N*C*HCHN β), 124.32 (N*C*HCHN α), 123.42 (NCH*C*HN β), 123.31 (NCH*C*HN α), 104.54 (C-1' β), 101.18 (C-1 β, C-1 α), 97.42 (C-1' α), 85.15, 82.98, 82.43, 81.13, 78.97, 78.72, 75.99, 75.87, 75.61, 75.44, 75.37, 75.12, 74.51, 74.49, 74.01, 73.73, 73.66, 73.49, 73.30, 73.04, 72.97, 71.21, 70.64, 70.30, 69.79, 69.71, 68.81, 67.27, 66.87, 47.99, 47.67, 36.83 (NCH_3), 30.36 ($CH_2CH_2CH_2$); **m/z** (ESI-HRMS) $C_{68}H_{69}N_2O_{14}^+$ ([M – OTf]$^+$) calculated: 1137.4743; found 1137.4731; **IR** ν_{max}/cm^{-1} 3067w, 2940w, 2862, 1730s (C=O), 1601, 1584w, 1496w, 1452, 1362, 1315, 1257s, 1224, 1153, 1092s, 1068s, 1029s, 853w, 740, 710s, 700, 638s, 622.

3-(3-Methylimidazolium)-1-propyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranoside trifluoromethanesulfonate 223



Following the general procedure for I-Tag glycosylations in flow using glycosyl acceptor 1-(3-hydroxypropyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate **155** (0.0348 g, 0.120 mmol, 1 eq) and glycosyl donor 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl trichloroacetimidate **159** (0.1529 g, 0.240 mmol, 2.0 eq). 0.60 mL of anhydrous acetonitrile was added to the donor/acceptor vial, resulting in a solution of volume 0.75 mL and therefore approximately 0.160 M in acceptor and 0.320 M in donor. To the other vial, 1.00 mL of anhydrous acetonitrile was added, followed by trimethylsilyl trifluoromethanesulfonate (10.9 μL, 0.060 mmol) to make a 0.06 M solution. Reaction solution was collected for a total of 10 min. The crude product was washed with water (3 mL), then the water was extracted with DCM (2 x 10 mL). The dried residue was washed with hexane:Et_2O 1:1 three times and hexane:Et_2O 1:3 three times then dried under reduced pressure to yield the title compound **223** as a single anomer (0.0590 g, 73 %); **^{1}H NMR** δ_H (500 MHz, Chloroform-*d*) 9.04 (1 H, s, NCHN), 7.38 – 7.22 (14 H, m, H_{arom}), 7.20 (2 H, dd, *J* 7.4, 1.9, H_{arom}), 6.89 (1 H, s, NC*H*CHN), 4.84 – 4.73 (3 H, m, 2 PhCH_2, H-2), 4.66 (1 H, d, *J* 11.3, PhC*H*H), 4.57 (2 H, d, *J* 10.9, PhCH_2), 4.50 (1 H, d, *J* 11.6, PhCH*H*), 4.33 (1 H, d, *J* 8.1, H-1), 4.31 – 4.26 (2 H, m, $OCH_2CH_2CH_2N$), 3.80 (3 H, s, NCH_3), 3.78 – 3.59 (6 H, m, H-3, H-4, H-6a, H-6b, $OCH_2CH_2CH_2N$), 3.53 – 3.45 (1 H, m, H-5), 2.13 (2 H, d, *J* 7.4, $OCH_2CH_2CH_2N$), 1.95 (3 H, s, $COCH_3$); **^{13}C NMR** δ_C (126 MHz, Chloroform-*d*) 169.98 (C=O), 138.09, 137.94, 137.84 (3 4° C_{arom}), 137.47 (NCHN), 128.68, 128.64, 128.56, 128.11, 128.09, 128.03, 127.99 (15 C_{arom}), 123.30 (NCH*C*HN), 122.79 (N*C*HCHN), 100.93 (C-1), 82.78, 77.89 (C-3, C-4), 75.47 (Ph*C*H_2), 75.22 (Ph*C*H_2), 74.77 (C-5), 73.61 (Ph*C*H_2), 73.20 (C-2), 68.84 (C-6), 66.14 ($OCH_2CH_2CH_2N$), 47.68 ($OCH_2CH_2CH_2N$), 36.52 (NCH_3), 30.14 ($OCH_2CH_2CH_2N$), 21.11 ($COCH_3$); **m/z** (ESI-HRMS) $C_{36}H_{43}N_2O_7^+$ ([M – OTf]$^+$) calculated: 615.3065; found 615.3055; **IR** ν_{max}/cm^{-1} 2925w, 2868w,

1740 (C=O), 1573w, 1497w, 1454, 1369, 1259s, 1225, 1154, 1088, 1057, 1030s, 915, 834w, 798w, 747, 700, 638s, 623; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ - 9 [*c* 0.67, DCM].

7. References

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8. Appendix

Publications contributed to during PhD studies:

R. Williams and M. C. Galan "Recent Advances in Organocatalytic Glycosylations" (Review), *Eur. J. Org. Chem.* 2017, **2017**, 6247-6264.

A. Sau, **R. Williams**, C. Palo-Nieto, A. Franconetti, S. Villar and M. C. Galan "Palladium-Catalysed Direct Stereoselective Synthesis of Deoxyglycosides from Glycals", *Angew. Chem. Int. Edit.* 2017, **56**, 3640-3644.

C. Palo-Nieto, A. Sau*, **R. Williams*** and M. C. Galan "Cooperative Brønsted Acid-Type Organocatalysis for the Stereoselective Synthesis of Deoxyglycosides", *J. Org. Chem.* 2017, **82**, 407−414.

*equal contribution

Presentations and Posters given during PhD studies:

I was selected to give a highly competitive presentation at the 20th European Carbohydrate Symposium "Eurocarb" international scientific conference in 2019, Leiden, Netherlands. The presentation was in support of the poster I presented at the conference, entitled "Continuous Flow Synthesis using Ionic Liquid Supports as an Expedient Method for Oligosaccharide Synthesis".

At the RSC Organic Division South-West Regional Meeting 2018, I presented a poster entitled: "Palladium-Catalysed Direct Stereoselective Synthesis of Deoxyglycosides from Glycals".